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(57) Abstract

Two new isolates of the Hepatitis C virus (HCV), J1 and J7, are disclosed. These new isolates comprise nucleotide and amino acid sequences which are distinct from the prototype HCV isolate, HCV1. Thus, J1 and J7 provide new polynucleotides and polypeptides for use, inter alia, in diagnostics, recombinant protein production and vaccine development.

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NEW HCV ISOLATES

Technical Field

The present invention relates to new isolates of the viral class Hepatitis C, polypeptides, polynucleotides and antibodies derived therefrom, as well as the use of such polypeptides, polynucleotides and antibodies in assays (e.g., immunoassays, nucleic acid hybridization assays, etc.) and in the production of viral polypeptides.

Background

Non-A, Non-B hepatitis (NANBH) is a transmissible disease or family of diseases that are believed to be viral-induced, and that are distinguishable from other forms of viral-associated liver diseases, including that caused by the known hepatitis viruses, i.e., hepatitis A virus (HAV), hepatitis B virus (HBV), and delta hepatitis virus (HDV), as well as the hepatitis induced by cytomegalovirus (CMV) or Epstein-Barr virus (EBV). NANBH was first identified

in transfused individuals. Transmission from man to chimpanzee and serial passage in chimpanzees provided evidence that NANBH is due to a transmissible infectious agent or agents. Epidemiologic evidence is suggestive that there may be three types of NANBH: the water-borne epidemic type; the blood or needle associated type; and the sporadically occurring (community acquired) type. However, until recently, no transmissible agent responsible for NANBH had not been identified.

Clinical diagnosis and identification of NANBH has been accomplished primarily by exclusion of other viral markers. Among the methods used to detect putative NANBH antigens and antibodies are agar-gel diffusion, counterimmunoelectrophoresis, immunofluorescence microscopy, immune electron microscopy, radioimmunoassay, and enzyme-linked immunosorbent assay. However, none of these assays has proved to be sufficiently sensitive, specific, and reproducible to be used as a diagnostic test for NANBH.

Until recently there has been neither clarity nor agreement as to the identity or specificity of the antigen antibody systems associated with agents of NANBH. It is possible that NANBH is caused by more than one infectious agent and unclear what the serological assays detect in the serum of patients with NANBH.

In the past, a number of candidate NANBH agents were postulated. See, e.g., Prince (1983) Ann. Rev. Microbiol. 37:217; Feinstone & Hoofnagle (1984) New Eng. J. Med. 311:185; Overby (1985) Curr. Heptol. 5:49; Overby (1986) Curr. Heptol. 6:65; Overby (1987) Curr. Heptol. 7:35; and Iwarson (1987) British Med. J. 295:946. However, there is no proof that any of these candidates represent the etiological agent of NANBH.

In 1987, Houghton et al. cloned the first virus definitively linked to NANBH. See, e.g., EPO Pub. No.

318,216; Houghton et al., Science 244:359 (1989). Houghton et al. described therein the cloning of an isolate from a new viral class, hepatitis C virus (HCV), the prototype isolate described therein being named "HCV1". HCV is a Flavi-like virus, with an RNA genome. Houghton et al. described the production of recombinant proteins from HCV sequences that are useful as diagnostic reagents, as well as polynucleotides useful in diagnostic hybridization assays and in the cloning of additional HCV isolates.

The demand for sensitive, specific methods for screening and identifying carriers of NANBH and NANBH contaminated blood or blood products is significant. Post-transfusion hepatitis (PTH) occurs in approximately 10% of transfused patients, and NANBH accounts for up to 90% of these cases. There is a frequent progression to chronic liver damage (25-55%).

Patient care as well as the prevention of transmission of NANBH by blood and blood products or by close personal contact require reliable diagnostic and prognostic tools to detect nucleic acids, antigens and antibodies related to NANBH. In addition, there is also a need for effective vaccines and immunotherapeutic therapeutic agents for the prevention and/or treatment of the disease.

While at least one HCV isolate has been identified which is useful in meeting the above needs, additional isolates, particularly those with divergent a genome, may prove to have unique applications.

Summary of the Invention

New isolates of HCV has been characterized from Japanese blood donors who have been implicated as NANBH carriers. These isolates exhibit nucleotide and amino acid sequence heterogeneity with respect to the prototype

isolate, HCV1, in several viral domains. It is believed that these distinct sequences are of in importance, particularly in diagnostic assays and in vaccine development.

In one embodiment, the present invention provides a DNA molecule comprising a nucleotide sequence of at least 15 bp from an HCV isolate substantially homologous to an isolate selected from the group J1 or J7, wherein said nucleotide sequence is distinct from the nucleotide sequence of HCV isolate HCV1.

In another embodiment, the present invention provides a DNA molecule comprising a nucleotide sequence of at least 15 bp encoding an amino acid sequence from a HCV isolate J1 or J7 wherein the J1 or J7 amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1.

Yet another embodiment of the present invention provides a purified polypeptide comprising an amino acid sequence from an HCV isolate substantially homologous to an isolate selected from the group J1 and J7, wherein said amino acid sequence is distinct from the sequence of the polypeptides encoded by the HCV isolate HCV1.

Still another embodiment of the present invention provides a polypeptide comprising an amino acid sequence from a HCV isolate J1 or J7 wherein the J1 or J7 amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1 and the polypeptide is immobilized on a solid support.

In a further embodiment of the present invention, an immunoassay for detecting the presence of anti-HCV antibodies in a test sample is provided comprising: (a) incubating the test sample under conditions that allow the formation of antigen-antibody complexes with an immunogenic polypeptide comprising an amino acid sequence from an HCV isolate substantially

homologous to an isolate selected from the group J1 and J7, wherein the amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1; and (b) detecting an antigen-antibody complex comprising the immunogenic polypeptide.

The present invention also provides a composition comprising anti-HCV antibodies that bind an HCV epitope substantially free of antibodies that do not bind an HCV epitope, wherein: (a) the HCV epitope comprises an amino acid sequence from an HCV isolate substantially homologous to an isolate selected from the group J1 and J7, wherein the amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1; and (b) the J1 or J7 amino acid sequence is not immunologically cross-reactive with HCV1.

A further embodiment of the present invention provides an immunoassay for detecting the presence of an HCV polypeptide in a test sample comprising: (a) incubating the test sample under conditions that allow the formation of antigen-antibody complexes with anti-HCV antibodies that bind an HCV epitope wherein: (i) the HCV epitope comprises an amino acid sequence from a HCV isolate J1 or J7; (ii) the J1 or J7 amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1; and (iii) the J1 or J7 amino acid sequence is not immunologically cross-reactive with HCV1; and (b) detecting an antigen-antibody complex comprising the anti-HCV antibodies.

Also provided by the present invention is a method of producing anti-HCV antibodies comprising administering to a mammal a polypeptide comprising an amino acid sequence from a HCV isolate J1 or J7 wherein the J1 or J7 amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1 whereby the mammal produces anti-HCV antibodies.

Yet another embodiment of the present invention provides a method of detecting HCV polynucleotides in a test sample comprising: (a) providing a probe comprising the DNA molecule of claim 1; (b) contacting the test sample and the probe under conditions that allow for the formation of a polynucleotide duplex between the probe and its complement in the absence of substantial polynucleotide duplex formation between the probe and non-HCV polynucleotide sequences present in the test sample; and (c) detecting any polynucleotide duplexes comprising the probe.

A still further embodiment of the present invention provides a method of producing a recombinant polypeptide comprising an HCV amino acid sequence, the method comprising: (a) providing host cells transformed by a DNA construct comprising a control sequences for the host cell operably linked to a coding sequence encoding an amino acid sequence from a HCV isolate J1 or J7 wherein the J1 or J7 amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1; (b) growing the host cells under conditions whereby the coding sequence transcribed and translated into the recombinant polypeptide; and (c) recovering the recombinant polypeptide.

These and other embodiments of the present invention will be readily apparent to those of ordinary skill in the art in view of the following description.

Brief Description of the Figures

Figure 1 shows the consensus sequence of the coding strand of a fragment from the J7 C/E domain with the heterogeneities.

Figure 2 shows the consensus sequence of the coding strand of a fragment from the J1 E domain with the heterogeneities.

Figure 3 shows the consensus sequence of the coding strand of a fragment of the J1 E/NS1 domain with the heterogeneities.

Figure 4 shows the consensus sequence of the coding strand of a fragment from the J1 NS3 domain with the heterogeneities.

Figure 5 shows the consensus sequence of the coding strand of a fragment from the J1 NS5 domain with the heterogeneities.

Figure 6 shows the homology of the J7 C/E consensus sequence with the nucleotide sequence of the same domain from HCV1.

Figure 7 shows the homology of the J1 E consensus sequence with the nucleotide sequence of the same domain from HCV1.

Figure 8 shows the homology of the J1 E/NS1 consensus sequence with the nucleotide sequence of the same domain from HCV1.

Figure 9 shows the homology of the J1 NS3 consensus sequence with the nucleotide sequence of the same domain from HCV1.

Figure 10 shows the homology of the J1 NS5 consensus sequence with the nucleotide sequence of the same domain from HCV1.

Figure 11 shows the putative genomic organization of the HCV1 genome.

Figure 12 shows the nucleotide sequence of the ORF of HCV1. In the figure nucleotide number 1 is the first A of the putative initiating methionine of the large ORF; nucleotides upstream of this nucleotide are numbered with negative numbers.

Figure 13 shows the consensus sequence of the coding strand of a fragment from the J1 NS1 domain (J1 1519) with the nucleotide sequence of the same domain

from HCV1. Also shown are the amino acids encoded therein.

Figure 14 shows a composite of the consensus sequence from the core to the NS1 domain of J1 with the nucleotide sequence of the same domain from HCV1. Also shown are the amino acids encoded therein.

Figure 15 shows a consensus sequence of the coding strand of the NS1 domain of J1, as determined in Example IV. Also shown are the nucleotide sequence of the same domain from HCV1, and the amino acids encoded in the HCV1 and J1 sequences.

Figure 16 shows a consensus sequence of a coding strand of the C200 region of the NS3-NS4 domain of J1. Also shown are the nucleotide sequence of the same domain from HCV1. Also shown are the amino acids encoded in the sequences.

Figure 17 shows a consensus sequence of the coding strand of the NS1 domain of J1, as determined in Example V. Also shown are the nucleotide sequence of the same domain from HCV1, and the amino acids encoded in the sequences.

Figure 18 shows a consensus sequence of the coding strand of the untranslated and core domains of J1. Also shown are the nucleotide sequence of the same domain from HCV1, and the amino acids encoded in the sequences.

Detailed Description of the Invention

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA techniques, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See e.g., Maniatis, Fitsch & Sambrook, MOLECULAR CLONING; A LABORATORY MANUAL

(1982); DNA CLONING, VOLUMES I AND II (D.N Glover ed. 1985); OLIGONUCLEOTIDE SYNTHESIS (M.J. Gait ed, 1984); NUCLEIC ACID HYBRIDIZATION (B.D. Hames & S.J. Higgins eds. 1984); TRANSCRIPTION AND TRANSLATION (B.D. Hames & S.J. Higgins eds. 1984); ANIMAL CELL CULTURE (R.I. Freshney ed. 1986); IMMOBILIZED CELLS AND ENZYMES (IRL Press, 1986); B. Perbal, A PRACTICAL GUIDE TO MOLECULAR CLONING (1984); the series, METHODS IN ENZYMOLOGY (Academic Press, Inc.); GENE TRANSFER VECTORS FOR MAMMALIAN CELLS (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory), Methods in Enzymology Vol. 154 and Vol. 155 (Wu and Grossman, and Wu, eds., respectively), Mayer and Walker, eds. (1987), IMMUNOCHEMICAL METHODS IN CELL AND MOLECULAR BIOLOGY (Academic Press, London), Scopes, (1987), PROTEIN PURIFICATION: PRINCIPLES AND PRACTICE, Second Edition (Springer-Verlag, N.Y.), and HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, VOLUMES I-IV (D.M. Weir and C. C. Blackwell eds 1986). All patents, patent applications, and other publications mentioned herein, both supra and infra, are hereby incorporated herein by reference.

The term "hepatitis C virus" has been reserved by workers in the field for an heretofore unknown etiologic agent of NANBH. Accordingly, as used herein, "hepatitis C virus" (HCV) refers to an agent causative of NANBH, which was formerly referred to as NANBV and/or BB-NANBV from the class of the prototype isolate, HCV1, described by Houghton et al. See, e.g., EPO Pub. No. 318,216 and U.S. Patent App. Serial No. 355,002, filed 19 May 1989 (available in non-U.S. applications claiming priority therefrom), the disclosures of which are incorporated herein by reference. The nucleotide sequence and putative amino acid sequence of HCV1 is shown in Figure 6. The terms HCV, NANBV, and BB-NANBV are used interchangeably herein. As an extension of this

terminology, the disease caused by HCV, formerly called NANB hepatitis (NANBH), is called hepatitis C. The terms NANBH and hepatitis C may be used interchangeably herein. The term "HCV", as used herein, denotes a viral species of which pathogenic strains cause NANBH, as well as attenuated strains or defective interfering particles derived therefrom.

HCV is a Flavi-like virus. The morphology and composition of Flavivirus particles are known, and are discussed by Brinton (1986) THE VIRUSES: THE TOGAVIRIDAE AND FLAVIVIRIDAE (Series eds. Fraenkel-Conrat and Wagner, vol eds. Schlesinger and Schlesinger, Plenum Press), p.327-374. Generally, with respect to morphology, Flaviviruses contain a central nucleocapsid surrounded by a lipid bilayer. Virions are spherical and have a diameter of about 40-50 nm. Their cores are about 25-30 nm in diameter. Along the outer surface of the virion envelope are projections that are about 5-10 nm long with terminal knobs about 2 nm in diameter.

The HCV genome is comprised of RNA. It is known that RNA containing viruses have relatively high rates of spontaneous mutation, i.e., reportedly on the order of 10^{-3} to 10^{-4} per incorporated nucleotide. Therefore, there are multiple strains, which may be virulent or avirulent, within the HCV class or species.

It is believed that the genome of HCV isolates is comprised of a single ORF of approximately 9,000 nucleotides to approximately 12,000 nucleotides, encoding a polyprotein similar in size to that of HCV1, an encoded polyprotein of similar hydrophobic and antigenic character to that of HCV1, and the presence of co-linear peptide sequences that are conserved with HCV1. In addition, the genome is believed to be a positive-stranded RNA.

Isolates of HCV comprise epitopes that are immunologically cross-reactive with epitopes in the HCV1 genome. At least some of these are epitopes unique to HCV when compared to other known Flaviviruses. The uniqueness of the epitope may be determined by its immunological reactivity with anti-HCV antibodies and lack of immunological reactivity with antibodies to other Flavivirus species. Methods for determining immunological reactivity are known in the art, for example, by radioimmunoassay, by ELISA assay, by hemagglutination, and several examples of suitable techniques for assays are provided herein.

It is also expected that the overall homology of HCV isolates and HCV1 genomes at the nucleotide level probably will be about 40% or greater, probably about 60% or greater, and even more probably about 80% to about 90% or greater. In addition that there are many corresponding contiguous sequences of at least about 13 nucleotides that are fully homologous. The correspondence between the sequence from a new isolate and the HCV1 sequence can be determined by techniques known in the art. For example, they can be determined by a direct comparison of the sequence information of the polynucleotide from the new isolate and HCV1 sequences. Alternatively, homology can be determined by hybridization of the polynucleotides under conditions which form stable duplexes between homologous regions (for example, those which would be used prior to S_1 digestion), followed by digestion with single-stranded specific nuclease(s), followed by size determination of the digested fragments.

Because of the evolutionary relationship of the strains or isolates of HCV, putative HCV strains or isolates are identifiable by their homology at the polypeptide level. Thus, new HCV isolates are expected to be more than about 40% homologous, probably more than

about 70% homologous, and even more probably more than about 80% homologous, and possibly even more than about 90% homologous at the polypeptide level. The techniques for determining amino acid sequence homology are known in the art. For example, the amino acid sequence may be determined directly and compared to the sequences provided herein. Alternatively the nucleotide sequence of the genomic material of the putative HCV may be determined, the amino acid sequence encoded therein can be determined, and the corresponding regions compared.

The ORF of HCV1 is shown in Figure 12. The non-structural, core, and envelope domains of the polyprotein have been predicted for HCV1 (Figure 5). The "C", or core, polypeptide is believed to be encoded from the 5′ terminus to about nucleotide 345 of HCV1. The putative "E", or envelope, domain of HCV1 is believed to be encoded from about nucleotide 346 to about nucleotide 1050. Putative NS1, or non-structural one domain, is thought to be encoded from about nucleotide 1051 to about nucleotide 1953. For the remaining domains, putative NS2 is thought to be encoded from about nucleotide 1954 to about nucleotide 3018, putative NS3 from about nucleotide 3019 to about nucleotide 4950, putative NS4 from about nucleotide 4951 to about nucleotide 6297, and putative NS5 from about nucleotide 6298 to the 3′ terminus respectively. The above boundaries are approximations based on an analysis of the ORF. The exact boundaries can be determined by those skilled in the art in view of the disclosure herein.

"HCV/J1" or "J1" and "HCV/J7" or "J7" refer to new HCV isolates characterized by the nucleotide sequence disclosed herein, as well as related isolates that are substantially homologous thereto; i.e., at least about 90% or about 95% at the nucleotide level. It is believed that the sequences disclosed herein characterize an HCV

subclass that is predominant in Japan and other Asian and/or Pacific rim countries. Additional J1 and J7 isolates can be obtained in view of the disclosure herein and EPO Pub. No. 318,216. In particular, the J1 and J7 nucleotide sequences disclosed herein, as well as the HCV1 sequences in Figure 12, can be used as primers or probes to clone additional domains of J1, J7, or additional isolates.

As used herein, a nucleotide sequence "from" a designated sequence or source refers to a nucleotide sequence that is homologous (i.e., identical) to or complementary to the designated sequence or source, or a portion thereof. The J1 sequences provided herein are a minimum of about 6 nucleotides, preferably about 8 nucleotides, more preferably about 15 nucleotides, and most preferably 20 nucleotides or longer. The maximum length is the complete viral genome.

In some aspects of the invention, the sequence of the region from which the polynucleotide is derived is preferably homologous to or complementary to a sequence which is unique to an HCV genome or the J1 and J7 genome. Whether or not a sequence is unique to a genome can be determined by techniques known to those of skill in the art. For example, the sequence can be compared to sequences in databanks, e.g., Genebank, to determine whether it is present in the uninfected host or other organisms. The sequence can also be compared to the known sequences of other viral agents, including those which are known to induce hepatitis, e.g., HAV, HBV, and HDV, and to other members of the Flaviviridae. The correspondence or non-correspondence of the derived sequence to other sequences can also be determined by hybridization under the appropriate stringency conditions. Hybridization techniques for determining the complementarity of nucleic acid sequences are known in

the art. See also, for example, Maniatis et al. (1982) MOLECULAR CLONING; A LABORATORY MANUAL (Cold Spring Harbor Press, Cold Spring Harbor, N.Y.). In addition, mismatches of duplex polynucleotides formed by hybridization can be determined by known techniques, including for example, digestion with a nuclease such as S1 that specifically digests single-stranded areas in duplex polynucleotides. Regions from which typical DNA sequences may be derived include, but are not limited to, regions encoding specific epitopes, as well as non-transcribed and/or non-translated regions.

The J1 of J7 polynucleotide is not necessarily physically derived from the nucleotide sequence shown, but may be generated in any manner, including for example, chemical synthesis or DNA replication or reverse transcription or transcription. In addition, combinations of regions corresponding to that of the designated sequence may be modified in ways known in the art to be consistent with an intended use. The polynucleotides may also include one or more labels, which are known to those of skill in the art.

An amino acid sequence "from" a designated polypeptide or source of polypeptides means that the amino acid sequence is homologous (i.e., identical) to the sequence of the designated polypeptide, or a portion thereof. An amino acid sequence "from" a designated nucleic acid sequence refers to a polypeptide having an amino acid sequence identical to that of a polypeptide encoded in the sequence, or a portion thereof. The J1 or J7 amino acid sequences in the polypeptides of the present invention are at least about 5 amino acids in length, preferably at least about 10 amino acids, more preferably at least about 15 amino acids, and most preferably at least about 20 amino acids.

The polypeptides of the present invention are not necessarily translated from a designated nucleic acid sequence; the polypeptides may be generated in any manner, including for example, chemical synthesis, or expression of a recombinant expression system, or isolation from virus. The polypeptides may include one or more analogs of amino acids or unnatural amino acids. Methods of inserting analogs of amino acids into a sequence are known in the art. The polypeptides may also include one or more labels, which are known to those of skill in the art.

The term "recombinant polynucleotide" as used herein intends a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is linked to a polynucleotide other than that to which it is linked in nature, or (2) does not occur in nature.

The term "polynucleotide" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA, and RNA. It also includes known types of modifications, for example, labels which are known in the art, methylation, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example proteins (including for e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators

(e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide.

"Purified polynucleotide" refers to a composition comprising a specified polynucleotide that is substantially free of other components, such composition typically comprising at least about 70% of the specified polynucleotide, more typically at least about 80%, 90% or even 95% to 99% of the specified polynucleotide.

"Purified polypeptide" refers to a composition comprising a specified polypeptide that is substantially free of other components, such composition typically comprising at least about 70% of the specified polypeptide, more typically at least about 80%, 90% or even 95% to 99% of the specified polypeptide.

"Recombinant host cells", "host cells", "cells", "cell lines", "cell cultures", and other such terms denote microorganisms or higher eukaryotic cell lines cultured as unicellular entities that can be, or have been, used as recipients for a recombinant vector or other transfer DNA, and include the progeny of the original cell which has been transformed. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

A "replicon" is any genetic element, e.g., a plasmid, a chromosome, a virus, a cosmid, etc. that behaves as an autonomous unit of polynucleotide replication within a cell; i.e., capable of replication under its own control.

A "cloning vector" is a replicon that can transform a selected host cell and in which another

polynucleotide segment is attached, so as to bring about the replication and/or expression of the attached segment. Typically, cloning vectors include plasmids, virus (e.g., bacteriophage vector) and cosmids.

An "integrating vector" is a vector that does not behave as a replicon in a selected host cell, but has the ability to integrate into a replicon (typically a chromosome) resident in the selected host to stably transform the host.

An "expression vector" is a construct that can transform a selected host cell and provides for expression of a heterologous coding sequence in the selected host. Expression vectors can be either a cloning vector or an integrating vector.

A "coding sequence" is a polynucleotide sequence which is transcribed into mRNA and/or translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5′-terminus and a translation stop codon at the 3′-terminus. A coding sequence can include, but is not limited to mRNA, cDNA, and recombinant polynucleotide sequences.

"Control sequence" refers to polynucleotide regulatory sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, control sequences generally include promoter, ribosomal binding site, and terminators. In eukaryotes generally control sequences include promoters, terminators and, in some instances, enhancers. The term "control sequences" is intended to include, at a minimum, all components the presence of which are necessary for expression, and may also include additional advantageous components.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

An "open reading frame" or ORF is a region of a polynucleotide sequence which encodes a polypeptide; this region may represent a portion of a coding sequence or a total coding sequence.

"Immunologically cross-reactive" refers to two or more epitopes or polypeptides that are bound by the same antibody. Cross-reactivity can be determined by any of a number of immunoassay techniques, such as a competition assay.

As used herein, the term "antibody" refers to a polypeptide or group of polypeptides which comprise at least one epitope. An "antigen binding site" is formed from the folding of the variable domains of an antibody molecule(s) to form three-dimensional binding sites with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows specific binding to form an antibody-antigen complex. An antigen binding site may be formed from a heavy- and/or light-chain domain (VH and VL, respectively), which form hypervariable loops which contribute to antigen binding. The term "antibody" includes, without limitation, chimeric antibodies, altered antibodies,univalent antibodies, Fab proteins, and single-domain antibodies. In many cases, the biding phenomena of antibodies to antigens is equivalent to other ligand/anti-ligand binding.

As used herein, a "single domain antibody" (dAb) is an antibody which is comprised of an HL domain,

which binds specifically with a designated antigen. A dAb does not contain a VL domain, but may contain other antigen binding domains known to exist to antibodies, for example, the kappa and lambda domains. Methods for preparing dAbs are known in the art. See, for example, Ward et al, Nature 341: 544 (1989).

Antibodies may also be comprised of VH and VL domains, as well as other known antigen binding domains. Examples of these types of antibodies and methods for their preparation and known in the art (see, e.g., U.S. Patent No. 4,816,467, which is incorporated herein by reference), and include the following. For example, "vertebrate antibodies" refers to antibodies which are tetramers or aggregates thereof, comprising light and heavy chains which are usually aggregated in a "y" configuration and which may or may not have covalent linkages between the chains. In vertebrate antibodies, the amino acid sequences of the chains are homologous with those sequences found in antibodies produced in vertebrates, whether in situ or in vitro (for example, in hybridomas). Vertebrate antibodies include, for example, purified polyclonal antibodies and monoclonal antibodies, methods for the preparation of which are described infra.

"Hybrid antibodies" are antibodies where chains are separately homologous with reference to mammalian antibody chains and represent novel assemblies of them, so that two different antigens are precipitable by the tetramer or aggregate. In hybrid antibodies, one pair of heavy and light chains are homologous to those found in an antibody raised against a first antigen, while a second pair of chains are homologous to those found in an antibody raised against a second antibody. This results in the property of "divalence", i.e., the ability to bind two antigens simultaneously. Such hybrids may also be formed using chimeric chains, as set forth below.

"Chimeric antibodies" refers to antibodies in which the heavy and/or light chains are fusion proteins. Typically, one portion of the amino acid sequences of the chain is homologous to corresponding sequences in an antibody derived from a particular species or a particular class, while the remaining segment of the chain is homologous to the sequences derived from another species and/or class. Usually, the variable region of both light and heavy chains mimics the variable regions or antibodies derived from one species of vertebrates, while the constant portions are homologous to the sequences in the antibodies derived from another species of vertebrates. However, the definition is not limited to this particular example. Also included is any antibody in which either or both of the heavy or light chains are composed of combinations of sequences mimicking the sequences in antibodies of different sources, whether these sources be from differing classes or different species of origin, and whether or not the fusion point is at the variable/constant boundary. Thus, it is possible to produce antibodies in which neither the constant nor the variable region mimic know antibody sequences. It then becomes possible, for example, to construct antibodies whose variable region has a higher specific affinity for a particular antigen, or whose constant region can elicit enhanced complement fixation, or to make other improvements in properties possessed by a particular constant region.

Another example is "altered antibodies", which refers to antibodies in which the naturally occurring amino acid sequence in a vertebrate antibody has been varies. Utilizing recombinant DNA techniques, antibodies can be redesigned to obtain desired characteristics. The possible variations are many, and range from the changing of one or more amino acids to the complete redesign of a

region, for example, the constant region. Changes in the constant region, in general, to attain desired cellular process characteristics, e.g., changes in complement fixation, interaction with membranes, and other effector functions. Changes in the variable region may be made to alter antigen binding characteristics. The antibody may also be engineered to aid the specific delivery of a molecule or substance to a specific cell or tissue site. The desired alterations may be made by known techniques in molecular biology, e.g., recombinant techniques, site-directed mutagenesis, etc.

Yet another example are "univalent antibodies", which are aggregates comprised of a heavy-chain/light-chain dimer bound to the Fc (i.e., stem) region of a second heavy chain. This type of antibody escapes antigenic modulation. See, e.g., Glennie et al. Nature 295: 712 (1982). Included also within the definition of antibodies are "Fab" fragments of antibodies. The "Fab" region refers to those portions of the heavy and light chains which are roughly equivalent, or analogous, to the sequences which comprise the branch portion of the heavy and light chains, and which have been shown to exhibit immunological binding to a specified antigen, but which lack the effector Fc portion. "Fab" includes aggregates of one heavy and one light chain (commonly known as Fab'), as well as tetramers containing the 2H and 2L chains (referred to as $F(ab)_2$), which are capable of selectively reacting with a designated antigen or antigen family. Fab antibodies may be divided into subsets analogous to those described above, i.e., "vertebrate Fab", "hybrid Fab", "chimeric Fab", and "altered Fab". Methods of producing Fab fragments of antibodies are known within the art and include, for example, proteolysis, and synthesis by recombinant techniques.

"Epitope" refers to an antibody binding site usually defined by a polypeptide, but also by non-amino acid haptens. An epitope could comprise 3 amino acids in a spatial conformation which is unique to the epitope, generally an epitope consists of at least 5 such amino acids, and more usually, consists of at least 8-10 such amino acids.

"Antigen-antibody complex" refers to the complex formed by an antibody that is specifically bound to an epitope on an antigen.

"Immunogenic polypeptide" refers to a polypeptide that elicits a cellular and/or humoral immune response in a mammal, whether alone or linked to a carrier, in the presence or absence of an adjuvant.

"Polypeptide" refers to a polymer of amino acids and does not refer to a specific length of the molecule. Thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not refer to or exclude post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

"Transformation", as used herein, refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for the insertion, for example, direct uptake, transduction, f-mating or electroporation. The exogenous polynucleotide may be maintained as a non-integrated vector, for example, a plasmid, or alternatively, may be integrated into the host genome.

A "transformed" host cell refers to both the immediate cell that has undergone transformation and its progeny that maintain the originally exogenous polynucleotide.

"Treatment" as used herein refers to prophylaxis and/or therapy.

"Individual", refers to vertebrates, particularly members of the mammalian species, and includes but is not limited to domestic animals, sports animals, and primates, including humans.

"Sense strand" refers to the strand of a double-stranded DNA molecule that is homologous to a mRNA transcript thereof. The "anti-sense strand" contains a sequence which is complementary to that of the "sense strand".

"Antibody-containing body component" refers to a component of an individual's body which is a source of the antibodies of interest. Antibody-containing body components are known in the art, and include but are not limited to, whole blood and components thereof, plasma, serum, spinal fluid, lymph fluid, the external sections of the respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, white blood cells, and myelomas.

"Purified HCV" isolate refers to a preparation of HCV particles which has been isolated from the cellular constituents with which the virus is normally associated, and from other types of viruses which may be present in the infected tissue. The techniques for isolating viruses are known to those of skill in the art, and include, for example, centrifugation and affinity chromatography.

An HCV "particle" is an entire virion, as well as particles which are intermediates in virion formation. HCV particles generally have one or more HCV proteins associated with the HCV nucleic acid.

"Probe" refers to a polynucleotide which forms a hybrid structure with a sequence in a target polynucleotide, due to complementarity of at least one region in the probe with a region in the target.

"Biological sample" refers to a sample of tissue or fluid isolated from an individual, including but not limited to, for example, whole blood and components thereof, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs, and also samples of in vitro cell culture constituents (including but not limited to conditioned medium resulting from the growth of cells in cell culture medium, putatively virally infected cells, recombinant cells, and cell components).

The invention pertains to the isolation and characterization of a newly discovered isolate of HCV, J1 and J7, their nucleotide sequences, their protein sequences and resulting polynucleotides, polypeptides and antibodies derived therefrom. Isolates J1 and J7 are novel in their nucleotide and amino acid sequences, and is believed to characteristic of HCV isolates from Japan and other Asian countries.

The nucleotide sequences derived from HCV/J1 and HCV/J7 are useful as probes to diagnose the presence of virus in samples, and to isolate other naturally occurring variants of the virus. These nucleotide sequences also make available polypeptide sequences of HCV antigens encoded within the J1 and J7 genome and permits the production of polypeptides which are useful as standards or reagents in diagnostic tests and/or as components of vaccines. Antibodies, both polyclonal and monoclonal, directed against HCV epitopes contained within these polypeptide sequences are also useful for diagnostic tests, as therapeutic agents, for screening of

antiviral agents, and for the isolation of the NANBH virus. In addition, by utilizing probes derived from the sequences disclosed herein it is possible to isolate and sequence other portions of the J1 and J7 genome, thus giving rise to additional probes and polypeptides which are useful in the diagnosis and/or treatment, both prophylactic and therapeutic, of NANBH.

The availability of the HCV/J1 and HCV/J7 nucleotide sequences enable the construction of polynucleotide probes and polypeptides useful in diagnosing NANBH due to HCV infection and in screening blood donors as well as donated blood and blood products for infection. For example, from the sequences it is possible to synthesize DNA oligomers of about 8-10 nucleotides, or larger, which are useful as hybridization probes to detect the presence of HCV RNA in, for example, sera of subjects suspected of harboring the virus, or for screening donated blood for the presence of the virus. The HCV/J1 and HCV/J7 sequences also allow the design and production of HCV specific polypeptides which are useful as diagnostic reagents for the presence of antibodies raised during NANBH. Antibodies to purified polypeptides derived from the HCV/J1 and HCV/J7 sequences may also be used to detect viral antigens in infected individuals and in blood.

Knowledge of these HCV/J1 and HCV/J7 sequences also enable the design and production of polypeptides which may be used as vaccines against HCV and also for the production of antibodies, which in turn may be used for protection against the disease, and/or for therapy of HCV infected individuals. Moreover, the disclosed HCV/J1 and HCV/J7 sequences enable further characterization of the HCV genome. Polynucleotide probes derived from these sequences, as well as from the HCV genome, may be used to screen cDNA libraries for additional viral cDNA

sequences, which, in turn, may be used to obtain additional overlapping sequences. See, e.g., EPO Pub. No. 318,216.

The HCV/J1 and HCV/J7 polynucleotide sequences, the polypeptides derived therefrom and the antibodies directed against these polypeptides, are useful in the isolation and identification of the BB-NANBV agent(s). For example, antibodies directed against HCV epitopes contained in polypeptides derived from the HCV/J1 sequences may be used in processes based upon affinity chromatography to isolate the virus. Alternatively, the antibodies may be used to identify viral particles isolated by other techniques. The viral antigens and the genomic material within the isolated viral particles may then be further characterized.

The information obtained from further sequencing of the HCV/J1 and HCV/J7 genome, as well as from further characterization of the HCV/J1 and HCV/J7 antigens and characterization of the genomes enable the design and synthesis of additional probes and polypeptides and antibodies which may be used for diagnosis, for prevention, and for therapy of HCV induced NANBH, and for screening for infected blood and blood-related products.

The availability of HCV/J1 and HCV/J7 cDNA sequences permits the construction of expression vectors encoding antigenically active regions of the polypeptide encoded in either strand. These antigenically active regions may be derived from coat or envelope antigens or from core antigens, or from antigens which are non-structural including, for example, polynucleotide binding proteins, polynucleotide polymerase(s), and other viral proteins required for the replication and/or assembly of the virus particle. Fragments encoding the desired polypeptides are derived from the cDNA clones

using conventional restriction digestion or by synthetic methods, and are ligated into vectors which may, for example, contain portions of fusion sequences such as beta-galactosidase or superoxide dismutase (SOD). Methods and vectors which are useful for the production of polypeptides which contain fusion sequences of SOD are described in EPO Pub. No. 196,056. Vectors encoding fusion polypeptides of SOD and HCV polypeptides are described in EPO Pub. No. 318,216. Any desired portion of the HCV cDNA containing an open reading frame, in either sense strand, can be obtained as a recombinant polypeptide, such as a mature or fusion protein. Alternatively, a polypeptide encoded in the cDNA can be provided by chemical synthesis.

The DNA encoding the desired polypeptide, whether in fused or mature form, and whether or not containing a signal sequence to permit secretion, may be ligated into expression vectors suitable for any convenient host. Both eukaryotic and prokaryotic host systems are presently used in forming recombinant polypeptides, and a summary of some of the more common control systems and host cell is given below. The polypeptide produced in such host cells is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use. Purification may be by techniques known in the art, for example, differential extraction, salt fractionation, chromatography on ion exchange resins, affinity chromatography, centrifugation, and the like. See, for example, Methods in Enzymology for a variety of methods for purifying proteins.

Such recombinant or synthetic HCV polypeptides can be used as diagnostics, or those which give rise to neutralizing antibodies may be formulated into vaccines. Antibodies raised against these polypeptides can also be

used as diagnostics, or for passive immunotherapy. In addition, antibodies to these polypeptides are useful for isolating and identifying HCV particles.

The HCV antigens may also be isolated from HCV virions. The virions may be grown in HCV infected cells in tissue culture, or in an infected host.

While the polypeptides of the present invention may comprise a substantially complete viral domain, in many applications all that is required is that the polypeptide comprise an antigenic or immunogenic region of the virus. An antigenic region of a polypeptide is generally relatively small--typically 8 to 10 amino acids or less in length. Fragments of as few as 5 amino acids may characterize an antigenic region. These segments may correspond to regions of HCV/J1 or HCV/J& epitopes. Accordingly, using the cDNAs of HCV/J1 and HCV/J7 as a basis, DNAs encoding short segments of HCV/J1 and HCV/J7 polypeptides can be expressed recombinantly either as fusion proteins, or as isolated polypeptides. In addition, short amino acid sequences can be conveniently obtained by chemical synthesis.

In instances wherein the synthesized polypeptide is correctly configured so as to provide the correct epitope, but is too small to be immunogenic, the polypeptide may be linked to a suitable carrier. A number of techniques for obtaining such linkage are known in the art, including the formation of disulfide linkages using N-succinimidyl-3-(2-pyridyl-thio)propionate (SPDP) and succinimidyl 4-(N-maleimido-methyl)cyclohexane-1-carboxylate (SMCC) obtained from Pierce Company, Rockford, Illinois, (if the peptide lacks a sulfhydryl group, this can be provided by addition of a cysteine residue.) These reagents create a disulfide linkage between themselves and peptide cysteine residues on one protein and an amide linkage through the epsilon-amino on

a lysine, or other free amino group in the other. A variety of such disulfide/amide-forming agents are known. See, for example, Immun. Rev. (1982) 62:185. Other bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of these thio-ether-forming agents are commercially available and include reactive esters of 6-maleimidocaproic acid, 2-bromoacetic acid, 2-iodoacetic acid, 4-(N-maleimido-methyl)cyclohexane-1-carboxylic acid, and the like. The carboxyl groups can be activated by combining them with succinimide or 1-hydroxyl-2-nitro-4-sulfonic acid, sodium salt. Additional methods of coupling antigens employs the rotavirus/"binding peptide" system described in EPO Pub. No. 259,149, the disclosure of which is incorporated herein by reference. The foregoing list is not meant to be exhaustive, and modifications of the named compounds can clearly be used.

Any carrier may be used which does not itself induce the production of antibodies harmful to the host. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins; polysaccharides, such as latex functionalized sepharose, agarose, cellulose, cellulose beads and the like; polymeric amino acids, such as polyglutamic acid, polylysine, and the like; amino acid copolymers; and inactive virus particles. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those skilled in the art.

In addition to full-length viral proteins, polypeptides comprising truncated HCV amino acid sequences encoding at least one viral epitope are useful immunological reagents. For example, polypeptides comprising such truncated sequences can be used as reagents in an immunoassay. These polypeptides also are

candidate subunit antigens in compositions for antiserum production or vaccines. While these truncated sequences can be produced by various known treatments of native viral protein, it is generally preferred to make synthetic or recombinant polypeptides comprising an HCV sequence. Polypeptides comprising these truncated HCV sequences can be made up entirely of HCV sequences (one or more epitopes, either contiguous or noncontiguous), or HCV sequences and heterologous sequences in a fusion protein. Useful heterologous sequences include sequences that provide for secretion from a recombinant host, enhance the immunological reactivity of the HCV epitope(s), or facilitate the coupling of the polypeptide to an immunoassay support or a vaccine carrier. See, e.g., EPO Pub. No. 116,201; U.S. Pat. No. 4,722,840; EPO Pub. No. 259,149; U.S. Pat. No. 4,629,783, the disclosures of which are incorporated herein by reference.

The size of polypeptides comprising the truncated HCV sequences can vary widely, the minimum size being a sequence of sufficient size to provide an HCV epitope, while the maximum size is not critical. In some applications, the maximum size usually is not substantially greater than that required to provide the desired HCV epitopes and function(s) of the heterologous sequence, if any. Typically, the truncated HCV amino acid sequence will range from about 5 to about 100 amino acids in length. More typically, however, the HCV sequence will be a maximum of about 50 amino acids in length, preferably a maximum of about 30 amino acids. It is usually desirable to select HCV sequences of at least about 10, 12 or 15 amino acids, up to a maximum of about 20 or 25 amino acids.

Truncated HCV amino acid sequences comprising epitopes can be identified in a number of ways. For example, the entire viral protein sequence can be

screened by preparing a series of short peptides that together span the entire protein sequence. By starting with, for example, 100-mer polypeptides, it would be routine to test each polypeptide for the presence of epitope(s) showing a desired reactivity, and then testing progressively smaller and overlapping fragments from an identified 100-mer to map the epitope of interest. Screening such peptides in an immunoassay is within the skill of the art. It is also known to carry out a computer analysis of a protein sequence to identify potential epitopes, and then prepare oligopeptides comprising the identified regions for screening. It is appreciated by those of skill in the art that such computer analysis of antigenicity does not always identify an epitope that actually exists, and can also incorrectly identify a region of the protein as containing an epitope.

The observed relationship of the putative polyproteins of HCV and the Flaviviruses allows a prediction of the putative domains of the HCV "non-structural" (NS) proteins. The locations of the individual NS proteins in the putative Flavivirus precursor polyprotein are fairly well-known. Moreover, these also coincide with observed gross fluctuations in the hydrophobicity profile of the polyprotein. It is established that NS5 of Flaviviruses encodes the virion polymerase, and that NS1 corresponds with a complement fixation antigen which has been shown to be an effective vaccine in animals. Recently, it has been shown that a flaviviral protease function resides in NS3. Due to the observed similarities between HCV and the Flaviviruses, deductions concerning the approximate locations of the corresponding protein domains and functions in the HCV polyprotein are possible. Figure 11 is a schematic of putative domains of the HCV polyprotein. The expression

of polypeptides containing these domains in a variety of recombinant host cells, including, for example, bacteria, yeast, insect, and vertebrate cells, should give rise to important immunological reagents which can be used for diagnosis, detection, and vaccines.

Although the non-structural protein region of the putative polyproteins of the HCV isolate described herein and of Flaviviruses appears to be generally similar, there is less similarity between the putative structural regions which are towards the N-terminus. In this region, there is a greater divergence in sequence, and in addition, the hydrophobic profile of the two regions show less similarity. This "divergence" begins in the N-terminal region of the putative NS1 domain in HCV, and extends to the presumed N-terminus. Nevertheless, it is still possible to predict the approximate locations of the putative nucleocapsid (N-terminal basic domain) and E (generally hydrophobic) domains within the HCV polyprotein. From these predictions it may be possible to identify approximate regions of the HCV polyprotein that could correspond with useful immunological reagents. For example, the E and NS1 proteins of Flaviviruses are known to have efficacy as protective vaccines. These regions, as well as some which are shown to be antigenic in the HCV1, for example those within putative NS3, C, and NS5, etc., should also provide diagnostic reagents.

The immunogenicity of the HCV sequences may also be enhanced by preparing the sequences fused to or assembled with particle-forming proteins such as, for example, hepatitis B surface antigen or rotavirus VP6 antigen. Constructs wherein the HCV epitope is linked directly to the particle-forming protein coding sequences produce hybrids which are immunogenic with respect to the HCV epitope. In addition, all of the vectors prepared

include epitopes specific to HBV, having various degrees of immunogenicity, such as, for example, the pre-S peptide. Thus, particles constructed from particle forming protein which include HCV sequences are immunogenic with respect to HCV and particle-form protein. See, e.g., U.S. Pat. No. 4,722,840; EPO Pub No. 175,261; EPO Pub. No. 259,149; Michelle et al. (1984) Int. Symposium on Viral Hepatitis.

Vaccines may be prepared from one or more immunogenic polypeptides derived from HCV/J1 or HCV/J7. The observed homology between HCV and Flaviviruses provides information concerning the polypeptides which are likely to be most effective as vaccines, as well as the regions of the genome in which they are encoded. The general structure of the Flavivirus genome is discussed in Rice et al. (1986) in THE VIRUSES: THE TOGAVIRIDAE AND FLAVIVIRIDAE (Series eds. Fraenkel-Conrat and Wagner, Vol eds. Schlesinger and Schlesinger, Plenum Press). The flavivirus genomic RNA is believed to be the only virus-specific mRNA species, and it is translated into the three viral structural proteins, i.e., C, M, and E, as well as two large nonstructural proteins, NV4 and NV5, and a complex set of smaller nonstructural proteins. It is known that major neutralizing epitopes for Flaviviruses reside in the E (envelope) protein. Roehrig (1986) in THE VIRUSES: THE TOGAVIRIDAE AND FLAVIVIRIDAE (Series eds. Fraenkel-Conrat and Wagner, Vol eds. Schlesinger and Schlesinger, Plenum Press). The corresponding HCV E gene and polypeptide encoding region may be predicted, based upon the homology to Flaviviruses. Thus, vaccines may be comprised of recombinant polypeptides containing epitopes of HCV E. These polypeptides may be expressed in bacteria, yeast, or mammalian cells, or alternatively may be isolated from viral preparations. It is also anticipated that the

other structural proteins may also contain epitopes which give rise to protective anti-HCV antibodies. Thus, polypeptides containing the epitopes of E, C, and M may also be used, whether singly or in combination, in HCV vaccines.

In addition to the above, it has been shown that immunization with NS1 (nonstructural protein 1), results in protection against yellow fever. Schlesinger et al (1986) J. Virol. 60:1153. This is true even though the immunization does not give rise to neutralizing antibodies. Thus, particularly since this protein appears to be highly conserved among Flaviviruses, it is likely that HCV NS1 will also be protective against HCV infection. Moreover, it also shows that nonstructural proteins may provide protection against viral pathogenicity, even if they do not cause the production of neutralizing antibodies.

In view of the above, multivalent vaccines against HCV may be comprised of one or more epitopes from one or more structural proteins, and/or one or more epitopes from one or more nonstructural proteins. These vaccines may be comprised of, for example, recombinant HCV polypeptides and/or polypeptides isolated from the virions. In particular, vaccines are contemplated comprising one or more of the following HCV proteins, or subunit antigens derived therefrom: E, NS1, C, NS2, NS3, NS4 and NS5. Particularly preferred are vaccines comprising E and/or NS1, or subunits thereof. In addition, it may be possible to use inactivated HCV in vaccines; inactivation may be by the preparation of viral lysates, or by other means known in the art to cause inactivation of Flaviviruses, for example, treatment with organic solvents or detergents, or treatment with formalin. Moreover, vaccines may also be prepared from attenuated HCV strains or from hybrid viruses such as

vaccinia vectors known in the art [Brown et al. Nature 319: 549-550 (1986)].

The preparation of vaccines which contain immunogenic polypeptide(s) as active ingredients is known to one skilled in the art. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified, or the protein encapsulated in liposomes. The active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1′-2′-dipalmitoyl-sn-glycerol-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against an immunogenic polypeptide containing an HCV antigenic sequence resulting from administration of this polypeptide in vaccines which are also comprised of the various adjuvants.

The vaccines are conventionally administered parenterally, by injection, usually, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10%-95% of active ingredient, preferably 25%-70%.

The proteins may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of 5 micrograms to 250 micrograms of antigen per dose, depends on the subject to be

treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered may depend on the judgment of the practitioner and may be peculiar to each subject.

The vaccine may be given in a single dose schedule, or preferably in a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may be with 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and or reenforce the immune response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the need of the individual and be dependent upon the judgment of the practitioner.

In addition, the vaccine containing the immunogenic HCV antigen(s) may be administered in conjunction with other immunoregulatory agents, for example, immune globulins.

The immunogenic polypeptides prepared as described above are used to produce antibodies, both polyclonal and monoclonal. If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is immunized with an immunogenic polypeptide bearing an HCV epitope(s). Serum from the immunized animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to an HCV epitope contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art, see for example, Mayer and Walker, eds. (1987) IMMUNOCHEMICAL METHODS IN CELL AND MOLECULAR BIOLOGY (Academic Press, London).

Monoclonal antibodies directed against HCV epitopes can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al. (1980) HYBRIDOMA TECHNIQUES; Hammerling et al. (1981), MONOCLONAL ANTIBODIES AND T-CELL HYBRIDOMAS; Kennett et al. (1980) MONOCLONAL ANTIBODIES; see also, U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,466,917; 4,472,500; 4,491,632; and 4,493,890. Panels of monoclonal antibodies produced against HCV epitopes can be screened for various properties; i.e., for isotype, epitope affinity, etc.

Antibodies, both monoclonal and polyclonal, which are directed against HCV epitopes are particularly useful in diagnosis, and those which are neutralizing are useful in passive immunotherapy. Monoclonal antibodies, in particular, may be used to raise anti-idiotype antibodies.

Anti-idiotype antibodies are immunoglobulins which carry an "internal image" of the antigen of the infectious agent against which protection is desired. Techniques for raising anti-idiotype antibodies are known in the art. See, e,g,, Grzych (1985), Nature 316:74; MacNamara et al. (1984), Science 226:1325, Uytdehaag et al (1985), J. Immunol. 134:1225. These anti-idiotype antibodies may also be useful for treatment and/or diagnosis of NANBH, as well as for an elucidation of the immunogenic regions of HCV antigens.

Using the HCV/J1 or HCV/J7 polynucleotide sequences as a basis, oligomers of approximately 8 nucleotides or more can be prepared, either by excision

or synthetically, which hybridize with the HCV genome and are useful in identification of the viral agent(s), further characterization of the viral genome(s), as well as in detection of the virus(es) in diseased individuals. The probes for HCV polynucleotides (natural or derived) are a length which allows the detection of unique viral sequences by hybridization. While 6-8 nucleotides may be a workable length, sequences of about 10-12 nucleotides are preferred, and about 20 nucleotides appears optimal. These probes can be prepared using routine methods, including automated oligonucleotide synthetic methods. Among useful probes, for example, are the clones disclosed herein, as well as the various oligomers useful in probing cDNA libraries, set forth below. A complement to any unique portion of the HCV genome will be satisfactory. For use as probes, complete complementarity is desirable, though it may be unnecessary as the length of the fragment is increased.

For use of such probes as diagnostics, the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids contained therein. The resulting nucleic acid from the sample may be subjected to gel electrophoresis or other size separation techniques; alternatively, the nucleic acid sample may be dot blotted without size separation. The probes are then labeled. Suitable labels, and methods for labeling probes are known in the art, and include, for example, radioactive labels incorporated by nick translation or kinasing, biotin, fluorescent probes, and chemiluminescent probes. The nucleic acids extracted from the sample are then treated with the labeled probe under hybridization conditions of suitable stringencies. Usually high stringency conditions are desirable in order to prevent false positives. The stringency of hybridization is determined by a number of factors dur-

ing hybridization and during the washing procedure, including temperature, ionic strength, length of time, and concentration of formamide. These factors are outlined in, for example, Maniatis, T. (1982) MOLECULAR CLONING; A LABORATORY MANUAL (Cold Spring Harbor Press, Cold Spring Harbor, N.Y.).

Generally, it is expected that the HCV genome sequences will be present in serum of infected individuals at relatively low levels, i.e., at approximately 10^2-10^3 chimp infectious doses (CID) per ml. This level may require that amplification techniques be used in hybridization assays. Such techniques are known in the art. For example, the Enzo Biochemical Corporation "Bio-Bridge" system uses terminal deoxynucleotide transferase to add unmodified 3'-poly-dT-tails to a DNA probe. The poly dT-tailed probe is hybridized to the target nucleotide sequence, and then to a biotin-modified poly-A. PCT App. No. 84/03520 and EPO Pub. No. 124,221 describe a DNA hybridization assay in which: (1) analyte is annealed to a single-stranded DNA probe that is complementary to an enzyme-labeled oligonucleotide; and (2) the resulting tailed duplex is hybridized to an enzyme-labeled oligonucleotide. EPO Pub. No. 204,510 describes a DNA hybridization assay in which analyte DNA is contacted with a probe that has a tail, such as a poly-dT tail, an amplifier strand that has a sequence that hybridizes to the tail of the probe, such as a poly-A sequence, and which is capable of binding a plurality of labeled strands.

A particularly desirable technique may first involve amplification of the target HCV sequences in sera approximately 10,000-fold, i.e., to approximately 10^6 sequences/ml. This may be accomplished, for example, by the polymerase chain reactions (PCR) technique described

which is by Saiki et al. (1986) Nature 324:163, Mullis, U.S. Patent No. 4,683,195, and Mullis et al. U.S. Patent No. 4,683,202. The amplified sequence(s) may then be detected using a hybridization assay which is described in co-pending European Publication No. 317-077 and Japanese application No. 63-260347, which are assigned to the herein assignee, and are hereby incorporated herein by reference. These hybridization assays, which should detect sequences at the level of 10^6/ml, utilize nucleic acid multimers which bind to single-stranded analyte nucleic acid, and which also bind to a multiplicity of single-stranded labeled oligonucleotides. A suitable solution phase sandwich assay which may be used with labeled polynucleotide probes, and the methods for the preparation of probes is described in EPO Pub. No. 225,807 which is hereby incorporated herein by reference.

The probes can be packaged into diagnostic kits. Diagnostic kits include the probe DNA, which may be labeled; alternatively, the probe DNA may be unlabeled and the ingredients for labeling may be included in the kit in separate containers. The kit may also contain other suitably packaged reagents and materials needed for the particular hybridization protocol, for example, standards, wash buffers, as well as instructions for conducting the test.

Both the HCV/J1 or HCV/J7 polypeptides which react immunologically with serum containing HCV antibodies and the antibodies raised against the HCV specific epitopes in these polypeptides are useful in immunoassays to detect presence of HCV antibodies, or the presence of the virus and/or viral antigens, in biological samples. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. An immunoassay for anti-HCV antibody

may utilize one viral epitope or several viral epitopes. When multiple epitopes are used, the epitopes may be derived from the same or different viral polypeptides, and may be in separate recombinant or natural polypeptides, or together in the same recombinant polypeptides.

An immunoassay for viral antigen may use, for example, a monoclonal antibody directed towards a viral epitope, a combination of monoclonal antibodies directed towards epitopes of one viral polypeptide, monoclonal antibodies directed towards epitopes of different viral polypeptides, polyclonal antibodies directed towards the same viral antigen, polyclonal antibodies directed towards different viral antigens or a combination of monoclonal and polyclonal antibodies.

Immunoassay protocols may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide. The labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known. Examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Typically, an immunoassay for anti-HCV antibody will involve selecting and preparing the test sample, such as a biological sample, and then incubating it with an antigenic (i.e., epitope-containing) HCV polypeptide under conditions that allow antigen-antibody complexes to form. Such conditions are well known in the art. In a heterogeneous format, the polypeptide is bound to a solid support to facilitate separation of the sample from the polypeptide after incubation. Examples of solid supports

that can be used are nitrocellulose, in membrane or microtiter well form, polyvinylchloride, in sheets or microtiter wells, polystyrene latex, in beads or microtiter plates, polyvinylidine fluoride, known as Immobulon™, diazotized paper, nylon membranes, activated beads, and Protein A beads. Most preferably, the Dynatech, Immulon™ 1 microtiter plate or the 0.25-inch polystyrene beads, which Spec finished by Precision Plastic Ball, are used in the heterogeneous format. The solid support is typically washed after separating it from the test sample. In a homogeneous format, the test sample is incubated with antigen in solution, under conditions that will precipitate any antigen-antibody complexes that are formed, as is know in the art. The precipitated complexes are then separated from the test sample, for example, by centrifugation. The complexes formed comprising anti-HCV antibody are then detected by any of a number of techniques. Depending on the format, the complexes can be detected with labeled anti-xenogeneic Ig or, if a competitive format is used, by measuring the amount of bound, labeled competing antibody.

In immunoassays where HCV polypeptides are the analyte, the test sample, typically a biological sample, is incubated with anti-HCV antibodies again under conditions that allow the formation of antigen-antibody complexes. Various formats can be employed, such as a "sandwich" assay where antibody bound to a solid support is incubated with the test sample; washed;incubated with a second, labeled antibody to the analyte; and the support is washed again. Analyte is detected by determining if the second antibody is bound to the support. In a competitive format, which can be either heterogeneous or homogeneous, a test sample is usually

incubated with and antibody and a labeled, competing antigen either sequentially or simultaneously. These and other formats are well known in the art.

The Flavivirus model for HCV allows predictions regarding the likely location of diagnostic epitopes for the virion structural proteins. The C, pre-M, M, and E domains are all likely to contain epitopes of significant potential for detecting viral antigens, and particularly for diagnosis. Similarly, domains of the nonstructural proteins are expected to contain important diagnostic epitopes (e.g., NS5 encoding a putative polymerase; and NS1 encoding a putative complement-binding antigen). Recombinant polypeptides, or viral polypeptides, which include epitopes from these specific domains may be useful for the detection of viral antibodies in infections blood donors and infected patients. In addition, antibodies directed against the E and/or M proteins can be used in immunoassays for the detection of viral antigens in patients with HCV caused NANBH, and in infectious blood donors. Moreover, these antibodies may be extremely useful in detecting acute-phase donors and patients.

Antigenic regions of the putative polyprotein can be mapped and identified by screening the antigenicity of bacterial expression products of HCV cDNAs which encode portions of the polyprotein. Other antigenic regions of HCV may be detected by expressing the portions of the HCV cDNAs in other expression systems, including yeast systems and cellular systems derived from insects and vertebrates. In addition, studies giving rise to an antigenicity index and hydrophobicity/hydrophilicity profile give rise to information concerning the probability of a region's antigenicity. Efficient detection systems may include

the use of panels of epitopes. The epitopes in the panel may be constructed into one or multiple polypeptides.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the polypeptides of the invention containing HCV epitopes or antibodies directed against HCV epitopes in suitable containers, along with the remaining reagents and materials required for the conduct of the assay (e.g., wash buffers, detection means like labeled anti-human Ig, labeled anti-HCV, or labeled HCV antigen), as well as a suitable set of assay instructions.

The HCV/J1 and HCV/J7 nucleotide sequence information described herein may be used to gain further information on the sequence of the HCV genomes, and for identification and isolation of additional HCV isolates related to J1 or J7. This information, in turn, can lead to additional polynucleotide probes, polypeptides derived from the HCV genome, and antibodies directed against HCV epitopes which would be useful for the diagnosis and/or treatment of HCV caused NANBH.

The HCV/J1 and HCV/J7 nucleotide sequence information herein is useful for the design of probes for the isolation of additional sequences which are derived from as yet undefined regions of the HCV genomes from which the J1 and J7 sequences are derived. For example, labeled probes containing a sequence of approximately 8 or more nucleotides, and preferably 20 or more nucleotides, which are derived from regions close to the 5′-termini or 3′-termini of the family of HCV cDNA sequences disclosed in the examples may be used to isolate overlapping cDNA sequences from HCV cDNA libraries. These sequences which overlap the cDNAs in the above-mentioned clones, but which also contain sequences derived from regions of the genome from which

the cDNA in the above mentioned clones are not derived, may then be used to synthesize probes for identification of other overlapping fragments which do not necessarily overlap the cDNAs described below. Methods for constructing cDNA libraries are known in the art. See, e.g. EPO Pub. No. 318,216. It is particularly preferred to prepare libraries from the serum of Japanese and other Asian patients diagnosed as having NANBH demonstrating antibody to HCV1 antigens; these are believed to be the most likely candidates for carriers of HCV/J1, HCV/J7, or related isolates.

HCV particles may be isolated from the sera from individuals with NANBH or from cell cultures by any of the methods known in the art, including for example, techniques based on size discrimination such as sedimentation or exclusion methods, or techniques based on density such as ultracentrifugation in density gradients, or precipitation with agents such as polyethylene glycol, or chromatography on a variety of materials such as anionic or cationic exchange materials, and materials which bind due to hydrophobicity.

A preferred method of isolating HCV particles or antigen is by immunoaffinity columns. Techniques for immunoaffinity chromatography are known in the art, including techniques for affixing antibodies to solid supports so that they retain their immunoselective activity. The techniques may be those in which the antibodies are adsorbed to the support (see, for example, Kurstak in ENZYME IMMUNODIAGNOSIS, page 31-37), as well as those in which the antibodies are covalently linked to the support. Generally, the techniques are similar to those used for covalent linking of antigens to a solid support, described above. However, spacer groups may be included in the bifunctional coupling agents so that the antigen binding site of the antibody remains accessible.

The antibodies may be monoclonal, or polyclonal, and it may be desirable to purify the antibodies before their use in the immunoassay.

The general techniques used in extracting the genome from a virus, preparing and probing a cDNA library, sequencing clones, constructing expression vectors, transforming cells, performing immunological assays such as radioimmunoassays and ELISA assays, for growing cells in culture, and the like are known in the art and laboratory manuals are available describing these techniques. However, as a general guide, the following sets forth some sources currently available for such procedures, and for materials useful in carrying them out.

Both prokaryotic and eukaryotic host cells may be used for expression of desired coding sequences when appropriate control sequences which are compatible with the designated host are used. Among prokaryotic hosts, E. coli is most frequently used. Expression control sequences for prokaryotes include promoters, optionally containing operator portions, and ribosome binding sites. Transfer vectors compatible with prokaryotic hosts are commonly derived from, for example, pBR322, a plasmid containing operons conferring ampicillin and tetracycline resistance, and the various pUC vectors, which also contain sequences conferring antibiotic resistance markers. These markers may be used to obtain successful transformants by selection. Commonly used prokaryotic control sequences include the Beta-lactamase (penicillinase) and lactose promoter systems (Chang et al.(1977), Nature 198:1056, the tryptophan (trp) promoter system (Goeddel et al. (1980) Nucleic Acid Res. 8:4057), and the lambda-derived P_L promoter and N gene ribosome binding site (Shimatake et al. (1981) Nature 292:128) and the hybrid tac promoter (De Boer et al. (1983) Proc.

Natl. Acad. Sci. USA 292:128) derived from sequences of the trp and lac UV5 promoters. The foregoing systems are particularly compatible with E. coli; if desired, other prokaryotic hosts such as strains of Bacillus or Pseudomonas may be used, with corresponding control sequences.

Eukaryotic hosts include yeast and mammalian cells in culture systems. Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Klebsiela lactis and Pichia pastoris are the most commonly used yeast hosts, and are convenient fungal hosts. Yeast compatible vectors carry markers which permit selection of successful transformants by conferring prototrophy to auxotrophic mutants or resistance to heavy metals on wild-type strains. Yeast compatible vectors may employ the 2 micron origin of replication (Broach et al. (1983) Math Enz. 101:307), the combination of CEN3 and ARS1 or other means for assuring replication, such as sequences which will result in incorporation of an appropriate fragment into the host cell genome. Control sequences for yeast vectors are known in the art and include promoters for the synthesis of glycolytic enzymes (Hess et al. (1968) J. Adv. Enzyme Eng. 7:149; Holland et al. (1978), J. Biol. Chem. 256:1385), including the promoter for 3 phosphoglycerate kinase (Hitzeman (1980), J. Biol. Chem. 255:2073). Terminators may also be included, such as those derived from the enolase gene (Holland (1981), J. Biol. Chem. 256:1385). Particularly useful control systems are those which comprise the glyceraldehyde-3 phosphate dehydrogenase (GAPDH) promoter or alcohol dehydrogenase (ADH) regulatable promoter, terminators also derived from GAPDH, and if secretion is desired, leader sequence from yeast alpha factor. In addition, the transcriptional regulatory region and the transcriptional initiation region which are operably

linked may be such that they are not naturally associated in the wild-type organism. These systems are described in detail in EPO Pub. No. 120,551; EPO Pub. No. 116,201; and EPO Pub. No. 164,556 all of which are incorporated herein by reference.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including HeLa cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, and a number of other cell lines. Suitable promoters for mammalian cells are also known in the art and include viral promoters such as that from Simian Virus 40 (SV40) (Fiers (1978), Nature 273:113), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Mammalian cells may also require terminator sequences and poly A addition sequences; enhancer sequences which increase expression may also be included, and sequences which cause amplification of the gene may also be desirable. These sequences are known in the art. Vectors suitable for replication in mammalian cells may include viral replicons, or sequences which insure integration of the appropriate sequences encoding NANBV epitopes into the host genome.

The vaccinia virus system can also be used to express foreign DNA in mammalian cells. To express heterologous genes, the foreign DNA is usually inserted into the thymidine kinase gene of the vaccinia virus and then infected cells can be selected. This procedure is known in the art and further information can be found in these references [Mackett et al. J. Virol. 49: 857-864 (1984) and Chapter 7 in DNA Cloning, Vol. 2, IRL Press].

In addition, viral antigens can be expressed in insect cells by the Baculovirus system. A general guide to baculovirus expression by Summer and Smith is A Manual

of Methods for Baculovirus Vectors and Insect Cell Culture Procedures (Texas Agricultural Experiment Station Bulletin No. 1555). To incorporate the heterologous gene into the Baculovirus genome the gene is first cloned into a transfer vector containing some Baculovirus sequences. THis transfer vector, when it is cotransfected with wild-type virus into insect cells, will recombine with the wild-type virus. Usually, the transfer vector will be engineered so that the heterologous gene will disrupt the wild-type Baculovirus polyhedron gene. This disruption enables easy selection of the recombinant virus since the cells infected with the recombinant virus will appear phenotypically different from the cells infected with the wild-type virus. The purified recombinant virus can be used to infect cells to express the heterologous gene. The foreign protein can be secreted into the medium if a signal peptide is linked in frame to the heterologous gene; otherwise, the protein will be bound in the cell lysates. For further information, see Smith et al Mol. & Cell. Biol. 3:2156-2165 (1983) or Luckow and Summers in Virology 17: 31-39 (1989).

Transformation may be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus and transducing a host cell with the virus, and by direct uptake of the polynucleotide. The transformation procedure used depends upon the host to be transformed. Bacterial transformation by direct uptake generally employs treatment with calcium or rubidium chloride (Cohen (1972), Proc. Natl. Acad. Sci. USA 69:2110; Maniatis et al. (1982), MOLECULAR CLONING; A LABORATORY MANUAL (Cold Spring Harbor Press, Cold Spring Harbor, N.Y.). Yeast transformation by direct uptake may be carried out using the method of Hinnen et al. (1978) Proc. Natl. Acad. Sci. USA 75: 1929. Mammalian

transformations by direct uptake may be conducted using the calcium phosphate precipitation method of Graham and Van der Eb (1978), Virology 52:546 or the various known modifications thereof.

Vector construction employs techniques which are known in the art. Site-specific DNA cleavage is performed by treating with suitable restriction enzymes under conditions which generally are specified by the manufacturer of these commercially available enzymes. The cleaved fragments may be separated using polyacrylamide or agarose gel electrophoresis techniques, according to the general procedures found in Methods in Enzymology (1980) 65:499-560. Sticky ended cleavage fragments may be blunt ended using E. coli DNA polymerase I (Klenow) in the presence of the appropriate deoxynucleotide triphosphates (dNTPs) present in the mixture. Treatment with S1 nuclease may also be used, resulting in the hydrolysis of any single stranded DNA portions.

Ligations are carried out using standard buffer and temperature conditions using T4 DNA ligase and ATP; sticky end ligations require less ATP and less ligase than blunt end ligations. When vector fragments are used as part of a ligation mixture, the vector fragment is often treated with bacterial alkaline phosphatase (BAP) or calf intestinal alkaline phosphatase to remove the 5′-phosphate and thus prevent religation of the vector; alternatively, restriction enzyme digestion of unwanted fragments can be used to prevent ligation. Ligation mixtures are transformed into suitable cloning hosts, such as E. coli, and successful transformants selected by, for example, antibiotic resistance, and screened for the correct construction.

Synthetic oligonucleotides may be prepared using an automated oligonucleotide synthesizer as

described by Warner (1984), DNA 3:401. If desired, the synthetic strands may be labeled with ^{32}P by treatment with polynucleotide kinase in the presence of ^{32}P-ATP, using standard conditions for the reaction. DNA sequences, including those isolated from cDNA libraries, may be modified by known techniques, including, for example site directed mutagenesis, as described by Zoller (1982), Nucleic Acids Res. 10:6487.

DNA libraries may be probed using the procedure of Grunstein and Hogness (1975), Proc. Natl. Acad. Sci. USA 73:3961. Briefly, in this procedure, the DNA to be probed is immobilized on nitrocellulose filters, denatured, and prehybridized with a buffer. The percentage of formamide in the buffer, as well as the time and temperature conditions of the prehybridization and subsequent hybridization steps depends on the stringency required. Oligomeric probes which require lower stringency conditions are generally used with low percentages of formamide, lower temperatures, and longer hybridization times. Probes containing more than 30 or 40 nucleotides such as those derived from cDNA or genomic sequences generally employ higher temperatures, e.g., about 40-42^{o}C, and a high percentage, e.g., 50%, formamide. Following prehybridization, 5'-^{32}P-labeled oligonucleotide probe is added to the buffer, and the filters are incubated in this mixture under hybridization conditions. After washing, the treated filters are subjected to autoradiography to show the location of the hybridized probe; DNA in corresponding locations on the original agar plates is used as the source of the desired DNA.

An enzyme-linked immunosorbent assay (ELISA) can be used to measure either antigen or antibody concentrations. This method depends upon conjugation of an enzyme to either an antigen or an antibody, and uses

the bound enzyme activity as a quantitative label. To measure antibody, the known antigen is fixed to a solid phase (e.g., a microplate or plastic cup), incubated with test serum dilutions, washed, incubated with anti-immunoglobulin labeled with an enzyme, and washed again. Enzymes suitable for labeling are known in the art, and include, for example, horseradish peroxidase. Enzyme activity bound to the solid phase is measured by adding the specific substrate, and determining product formation or substrate utilization colorimetrically. The enzyme activity bound is a direct function of the amount of antibody bound.

To measure antigen, a known specific antibody is fixed to the solid phase, the test material containing antigen is added, after an incubation the solid phase is washed, and a second enzyme-labeled antibody is added. After washing, substrate is added, and enzyme activity is estimated colorimetrically, and related to antigen concentration.

Examples

I

This example describes the cloning of the HCV/J1 and HCV/J7 nucleotide sequences.

Both blood samples which were used as a source of HCV virions were found to be positive in an anti-HCV antibody assay. THe HCV isolates from these samples were named HCV/J1 and HCV/J7. The infectivity of the blood sample containing the J1 isolate was confirmed by a prospective study of blood transfusion recipients. Dr. Tohru Katayama from the Department of Surgery at the National Tokyo Chest Hospital collected blood from patients who have contracted post-transfusion non-A, non-B hepatitis. He also collected blood samples from the respective blood donors of these patients. Next, these

samples were assayed for antibodies to the C100-3 HCV1 antigen (EPO Pub. No. 318,216), and blood from one of the donors was found to be positive.

Isolation of the RNA from the blood samples began by pelleting virions in the blood sample by ultracentrifugation [Bradley, D.W., McCaustland, K.A., Cook E.H., Schable, C.A., Ebert, J.W. and Maynard, J.E. (1985) Gastroenterology 88, 773-779]. RNA was then extracted from the pellet by the guanidinium/cesium chloride method [Maniatis T., Fritsch, E.F., and Sambrook J. (1982) "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor] and further purified by phenol/chloroform extraction in the presence of urea, [Berk, A.J. Lee,F., Harrison, T., Williams, J. and Sharp, P.A. (1979) Cell 17, 935-944].

Five pairs of synthetic oligonucleotide primers were designed from the C/E, E, E/NS1, NS3, and NS5 domains of the nucleotide sequence of HCV1 to isolate fragments from the J1 and J7 genome. The first set of primers were to isolate the sequence from the core and some of the envelope domain. The second set of primers were to isolate the sequences in the envelope domain. The third set of primers were to isolate a fragment which overlapped the putative envelope and non-structural one, NS1, domains. The fourth and fifth set of primers were used to isolate fragments from non-structural domains three and five, NS3 and NS5. The sequences for the various primers are shown below:

The sequence of the primers for the C/E region were:

21S	5′ CGTGCCCCCGCAAGACTGCT 3′
J80A	5′ CCGTCCTCCAGAACCCGGAC 3′

The sequence of the primers for the E region were:

71S 5′ GCCGACCTCATGGGGTACAT 3′

J132A 5′ AACTGCGACACCACTAAGGC 3′

The sequence of the primers for the E/NS1 region were:

127S 5′ TGGCATGGGATATGATGATG 3′

166A 5′ TTGAACTTGTGGTGATAGAA 3′

The sequence of the primers for the NS3 region were :

464S 5′ GGCTATACCGGCGACTTCGA 3′

526A 5′ GACATGCATGTCATGATGTA 3′

The sequence of the primers for the NS5 region were:

870S 5′ GCTGGAAAGAGGGTCTACTA 3′

917A 5′ GTTCTTACTGCCCAGTTGAA 3′

1 μg of the antisense primers, 166A, 526A, or 917A, was added to 10 units of reverse transcriptase (Biorad) to synthesize cDNA fragments from the isolated RNA as the template. The cDNA fragments were then amplified by a standard polymerase chain reaction [Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn G.T., Erlich, H.A., and Arnheim, N. (1985) Science 230, 1350-1354] after 1 μg of the appropriate sense primer, 21S, 71S, 127S, 464S or 870S, was added.

The cDNA fragments amplified by the PCR method were gel isolated and cloned by blunt-end ligation into the SmaI site of pUC119 [Vieira, J. and Messing, J. (1987) Methods in Enzymology 153, 3-11] or into the SnaBI site of charomid SB, a derivative of the cloning vector charomid 9-42 [Saito, I. and Stark, G. (1986) Proc. Natl. Acad. Sci. USA 83: 8664-8668]. Clones which contain the fragments of the five viral domains were successfully constructed.

From the PCR reaction of the Japanese isolates, J1 and J7, three independent clones from each region, C/E, E, E/NS1, NS3, and NS5, have been sequenced by the dideoxy chain termination method.

Sequence from all regions except C/E has been isolated from the J1 isolate. Sequence from only the C/E region has been isolated from the J7 isolate. Surprisingly, fragments isolated from both isolates are neither longer or shorter than what would be predicted from the HCV1 genome. However, there is heterogeneity between clones containing sequence from the same region. Consequently, a consensus sequence was constructed for each of the domains, C/E, E, E/NS1, NS3 and NS5, as shown respectively in Figures 1 through 5. These differences may be explained as artifacts which occur randomly during the PCR amplification [Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., and Arnheim, N. (1985) Science 230, 1350-1354]. Another explanation is that more than one virus genome is present in the plasma of a single healthy carrier and that these genomes are heterogeneous at the nucleotide level.

To clarify this point, it was determined how many of these nucleotide differences would lead to amino acid changes, using the sequence from the NS3 domain of the J1 isolate as an example. Out of the five nucleotide differences, three fall on the third position of the amino acid codon and do not change the amino acid sequence. Both of the remaining two nucleotide changes fall on the first position of the amino acid codon and generate amino acid changes of threonine to alanine and proline to alanine, all of which are small, neutral amino acid residues. Similarly, when analyzing the nucleotide differences in other domains, many silent and conserved

mutations are found. These results suggest that nucleotide sequences of the HCV genomes in the plasma of a single healthy donor are heterogeneous at the nucleotide level.

In addition, once the consensus sequences for each of the fragments were compiled each sequence was compared to the HCV1 isolate in Figures 6 through 10. In Figure 6 the fragment from the C/E region of the J7 isolate shows a 92.8%, 512/552, nucleotide and 97.4%, 150/154, amino acid homology to the HCV1 isolate. The fragment from the E domain of J1 shows a slightly lower nucleotide and amino acid homology to HCV1 in Figure 7 of 76.2% and 82.9%, respectively. The fragment from the J1 isolate which overlaps the envelope and non-structural one domains shows the lowest homology to HCV1, as seen in Figure 8, where the J1 isolate has a 71.5% nucleotide homology and a 73.5% amino acid homology to HCV1. Figure 9 shows a comparison of the fragment from the NS3 domain of J1 to HCV1. The homology between the nucleotides sequences is 79.8%, while the amino acid homology between the isolates is quite high, 92.2% or 179/194 amino acids. Figure 10 shows the homology between the NS5 sequences from J1 and HCV1. The sequences have a 84.3% nucleotide and 88.7% amino acid homology.

The vectors described in the examples above were deposited with the Patent Microorganism Depository, Fermentation Institute, Agency of Industrial Science and Technology at 1-3, Higashi 1-chome Tsukuba-chi, Ibaragi-ken 305, Japan, and will be maintained under the provisions of the Budapest Treaty. The accession numbers and dates of the deposit are listed below, on page 68.

III

An HCV/J1 clone, J1-1519, was isolated using the essentially the techniques described above. However, the primers used in the isolation were J159S and 199A.

The sequences of the oligomeric primers J159S and 199A, which follow, were based upon those in J1-1216 and in HCV1.

J159S 5′ ACT GCC CTG AAC TGC AAT GA 3′

199A 5′ AAT CCA GTT GAG TTC ATC CA 3′

Clone J1-1519 is comprised of an HCV cDNA sequence of 367 nucleotides which spans most of the 5′-half of the NS1 region and which overlaps the E-region clone, J1-1216, by 31 nucleotides. Three independent clones spanning this region were sequenced; the sequences in this region obtained from the three clones were identical. The sequence of the HCV cDNA in J1-1216 (shown in the figure as J1) and the amino acids encoded therein (shown above the nucleotide sequence) are shown in Figure 13. Figure 13 also shows the sequence differences between J1-1216 in the comparable region of the prototype HCV1 cDNA (indicated in the figure as PT), and the resulting changes in the encoded amino acids. The homology between the J1-1216 and HCV1 cDNA is approximately 70% at the nucleotide level, and about 75% at the amino acid level.

A composite of the sequences from the putative core to NS1 region of the J1 isolate is shown in Figure 14; also shown in the figure are the amino acids encoded in the J1 sequence. The variation from the HCV1 prototype sequence is shown in the line below the J1 nucleotide sequence; the dashed lines indicate homologous sequences. The nonhomologous amino acid encoded in the HCV1 prototype sequence is shown below the HCV1 nucleotide sequence.

Cloned material containing the J1/1519 HCV cDNA (pS1-1519) has been maintained in DH5α, and deposited with the Patent Microorganism Depository.

IV

Several regions of the J1 isolate, including the C200-C100 region from the putative NS3-NS4 region (which encompasses the region encoding the 5-1-1 polypeptide in HSV1 (See EPO Pub. No. 318,216), and the putative NS1 - E region, were amplified using the PCR method. The C200-C100 region includes nucleotides 3799 to 5321 of the prototype HCV1. RNA was extracted as described above, except that extraction was with guanidinium thiocyanate in the presence of Proteinase K and sodium dodecylsulfate (SDS) (Maniatis (1982), supra). The RNA was transcribed into HCV cDNA by incubation in a 25 μl reaction comprised of 1 μM of each primer, 40 units of RNase inhibitor (RNASIN), 5 units of AMV reverse transcriptase, and salts and buffer necessary for the reaction. Amplification of a segment of the HCV cDNA from the designated region was performed utilizing pairs of synthetic oligomer 16-mer primers. PCR amplification was accomplished in three rounds (PCR I, PCR II, and PCRIII). The second and third rounds of PCR amplification (PCR II) utilized different sets of PCR primers; the first PCR reaction was diluted 10-fold and multiple rounds of PCR amplification were carried out with the new primers, so that ultimately up to 50% of the products of the first PCR reaction (PCR I) were reamplified. The primers used for the amplification of the regions were the following. These primers, with the exception of J1C200-3 which was derived from the J1 isolate sequence, were derived from the prototype HCV1 sequence.

Primers for amplification of the "5-1-1" region from NS3-NS4.

PCR I

511/16A (sense, derived from nucleotides starting at number 1528 of HCV1)

5′ AAC AGG CTG CGT GGT C 3′

511/16B (anti-sense, derived from nucleotides ending at 5260 of HCV1)

5′ AGT TGG TCT GGA CAG C 3′

PCR II

511/35A (sense, the HCV portion derived from nucleotides starting at number 5057 of HSV1; the restriction enzyme site is underlined)

5′ CTTGAATTC TCG TCT TGT CCG GGA AGC CGG CAA TC 3′

511/35B (anti-sense, the HCV portion derived from nucleotides ending at number 5233 of HSV1; the restriction enzyme site is underlined)

5′ CTTGAATTC CCT CTG CCT GAC GGG ACG CGG TCT GC 3′

PCRIII

511/35A (see supra)

VSNrc7 (antisense, derived from nucleotides ending at number 5804 of HSV1)

5′ GTA GTG CGT GGG GGA AAC AT 3′

Primers for amplification of the "NS1/E" region

PCR I

J1(E2)3 (sense, the HCV portion derived from nucleotides starting at number 953 of HSV1, the restriction enzyme site is underlined)

5′ CTTAGAATTC TGG CAT GGG ATA TGA TGA TG 3′

J1(E)4 (sense, the HCV portion derived from nucleotides starting at number 1087 of HSV1, the restriction enzyme site is underlined)

5′ CTTAGAATTC TCC ATG GTG GGG AAC TGG GC 3′

J1rc12 (anti-sense, the HCV portion derived from nucleotides ending at 1995 of HSV1, the restriction enzyme site is underlined)

5′ CTTGAATTC TAA CGG GCT GAG CTC GGA 3′

J1rc13 (anti-sense, the HCV portion derived from nucleotides ending at 1941 of HSV1, the restriction enzyme site is underlined)

5′ CTTAGAATTC CGT CCA GTT GCA GGC AGC TTC 3′

PCR II

J1rc13 (see supra)

J1IZ-1 (sense, the HCV portion is derived from nucleotides starting at number 1641 of HCV1, the restriction enzyme site is underlined)

5′ CTTGAATTC CAA CTG GTT CGG CTG TAC A 3′

J1IZ-2 (sense, the HCV portion is derived from nucleotides starting at number 1596 of HCV1, the restriction enzyme site is underlined)

5′ TGA GAC GGA CGT GCT GCT CCT 3′

Primers for the C200-C100 region of the "NS3-NS4" region

PCR I

J1C200-1 (sense, derived from nucleotides starting at number 3478 of HCV1)

5′ TCC TAC TTG AAA GGC TC 3′

J1C200-3 (anti-sense, derived from nucleotides ending at number 4402 of HCV1)

5′ GGA TCC AAG CTG AAA TCG AC 3′

J1rc52 (anti-sense, the HCV portion derived from nucleotides ending at 5853 of HCV1, the restriction enzyme site is underlined)

5′ CTTAGAATTC GAG GCT GCT GAG ATA GGC AGT 3′

511/16A (see above).

PCR II

J1C200-2 (sense, the HCV portion derived from nucleotides starting at number 3557 of HCV1, the restriction enzyme site is underlined)

5′ CTTGAATTC CCC GTG GAG TGG CTA AGG CGG TGG ACT 3′

J1C200-4 (anti-sense, the HCV portion derived from nucleotides ending at 4346 of HCV1, the restriction enzyme site is underlined)

5′ CTTGAATTC TCG AAG TCG CCG GTA TAG CCG GTC ATG 3′

511/35A (see above)

J1rc51 (anti-sense, the HCV portion derived from nucleotides ending at 5826 of HCV1, the restriction enzyme site is underlined)

5′ CTTAGAATTC GGC AGC TGC ATC GCT CTC CGG CAC 3′

The amplified HCV cDNAs were either sequenced directly without cloning, and/or were cloned. Sequencing was accomplished using an assymetric PCR technique, essentially as described in Shyamala and Ames, J. Bacteriology 171:1602 (1989). In this technique, amplification of the cDNA is carried out with a limiting concentration of one of the primers (usually in a ratio of about 1:50) in order to get preferential amplification of one strand. The preferentially amplified strand is then sequenced by the dideoxy chain termination method.

The primers used for assymetric sequencing by the PCR method were the following. For the NS1 region: J11Z-1 and J1rc13 (sequenced with both); J11Z-2, J1rc13 (confirmed on both strands). For the NS3-NS4 region, which includes the C200-C100 N-terminal region, C200-C100 C-terminal region, and the 5-1-1 region: J1C200-2 and J1C200-7 (for the N-terminal region of C200-C100), and J1C200-4 and J1C200-6 (for the C200-C100 C-terminal region); and 511/35A and hep 4 (for the 5-1-1 region). The sequences for J1C200-2, J1C200-4, and 511/35A are shown supra; the sequences of hep 4, J1C200-6, and J1C200-7 are the following.

hep 4 (derived from nucleotides starting at number 5415 of HCV1)

5′ TT GGC TAG TGG TTA GTG GGC TGG TGA CAG 3′

J1C200-6 (the HCV portion derived from nucleotides starting at number 3875 of HCV1, the restriction enzyme site is underlined)

5′ CTTGAATTC CGT ACT CCA CCT ACG GCA AGT TCC TT 3′

J1C200-7 (the HCV portion derived from nucleotides starting at number 3946 of HCV1, the restriction enzyme site is underlined)

5′ CTTGAATTC GTG GCA TCC GTG GAG TGG CAC TCG TC 3′

The sequences obtained by assymetric sequencing of the "NS1" region, the C200-C100 region, and the 5-1-1 region are shown in Figure 15, and Figure 16, respectively. In the figures, the amino acids encoded in the J1 sequence are shown above the J1 nucleotide sequence. The differences between the J1 sequence and the HCV1 prototype nucleotide sequence is shown below the J1 sequence (the dashes indicate homologous nucleotides in both sequences). The encoded amino acids which differ in the HCV1 prototype sequence are shown below the HCV1 nucleotide sequence.

HCV cDNAs from the NS1 region, the C200-C100 region, and the 5-1-1 region were cloned. A 300 bp and a 230 bp fragment from the putative NS1 region, were cloned into a derivative of the commercially available vector, pGEM-3Z, in host HB101, and deposited with the ATCC as AW-300bp. The derivative vectors maintain the original pGEM-3Z polylinkers, an intact Amp^r gene, and the genes required for replication in E. coli. The HCV cDNA fragments may be removed with SacI and XbaI. HCV cDNAs containing 770 bp N-terminal fragments of C200 were cloned into pM1E in HB101, 12 clones were pooled and deposited with the ATCC as AW-770bp-N; the HCV cDNA may be removed from the vector with HaeII. The resultant HaeII fragment will contain vector DNA of 300 bp and 250 bp at the 5′ and 3′ ends, respectively. HCV cDNAs containing 700 bp C-terminal fragments of C200 (AW-700bp-C) were cloned into M13mp10 and maintained in host DH5α-F′; cloning was into the vector polylinker site. The resultant phage were pooled, and deposited with the ATCC on September 11, 1990 as AW-700bp-N or AW-700bp-C.

HCV cDNA from J1 equivalent to the 5-1-1 region of HCV1 was cloned into mp19 R1 site, and maintained in DH5α-F′. Several m13 phage superanants from this cloning were pooled and deposited with the ATCC as J1 5-1-1, on September 11, 1990. The HCV cDNAs may be obtained from the phage by treatment with EcoRI. Accession numbers for J1 5-1-1 and AW-700bp-N or AW-700bp-C may be obtained by telephoning the ATCC at (301) 881-2600.

The above-described cloned material was deposited with the American Type Culture Collection (ATCC).

V

An HCV cDNA library containing sequences of the putative "NS1" region of the J1 isolate was created by directional cloning in λ-gt22. The "NS1" region extends from about nucleotide 1460 to about nucleotide 2730 using the numbering system of the HCV1 prototype nucleic acid sequence, where nucleotide 1 is the first nucleotide of the initiating methionine codon for the putative polyprotein. The cloning was accomplished using essentially the method described by Han and Rutter in GENETIC ENGINEERING, Vol 10 (J.K. Setlow, Ed., Plenum Publishing Co., 1988), except that the primers for the synthesis of the first and second strand of HCV cDNA were JHC67 and JHC68, respectively, and the source of RNA was the J1 plasma. In this method the RNA is extracted with guanidium thiocyanate at a low temperature. The RNA is then converted to full length cDNA, which is cloned in a defined orientation relative to the lacZ promoter in λ-phage. Using this method, the HCV cDNAs to J1 RNA were inserted into the NotI site of λ-gt22. The presence of "NS1" sequences in the library was detected using as probe, A1x54.

The sequence of a region of "NS1" downstream from the region shown in Figure 14, but which overlaps the region by about 20 nucleotides, was determined using the assymetric sequencing technique described above, but substituting as primers for PCR amplification, Alx 61 and Alx 62. The resulting sequence is shown in Figure 17. (It should be noted that the PCR amplification was of a region from about nucleotide 1930 to about nucleotide 2340; this region is also encompassed in the sequence shown in Figure 15). The sequences of the primers and probes used to obtain the HCV cDNA library in λ-gt22, and to sequence the portion of the "NS1" region were the following.

JHC 67

5′ GACGC GGCCG CCTCC GTGTC CAGCG CGT 3′

JHC 68

5′ CGTGC GGCCG CAAGA CTGCT AGCCG AGGT 3′

ALX 61

5′ ACCTG CCACT GTGTA GTGGT CAGCA GTAAC 3′

ALX 62

5′ ACGGA CGTCT TCGTC CTTAACAATA CCAGG 3′

ALX 54

5′ GAACT TTGCG ATCTG GAAGACAGGG ACAGG 3′

A 400 bp fragment of J1 HCV cDNA derived from the sequenced region was cloned into pGEM3z and maintained in HB101; the HCV cDNA may be removed from the vector with SacI and XbaI. Host cells transformed with the vector (JH-400bp) have been deposited with the ATCC.

A pooled cDNA library was created from the J1 serum; the pooled library spans the J1 genome and is identified as HCV-J1 λ gt22. The pooled cDNA library was created by pooling aliquots of 11 individual cDNA libraries, which had been prepared using the directional cloning technique described above, except that the libraries were created from primers which were designed

to yield HCV cDNAs which spanned the genome. The primers were derived from the sequence of HCV1, and included JHC 67 and JHC 68. The HCV cDNAs were inserted into the NotI site of λ-gt22. The pooled cDNA library, HCV-J1 λ gt22, has been deposited with the ATCC.

VI

The sequence of a region of the polynucleotide upstream of that shown in Figure 14 was determined. This region begins at nucleotide -267 with respect to the HCV-1 (See Figure 12) and extends for 560 nucleotides. Sequencing was accomplished by preparing HCV cDNA from RNA extracted from J1 serum, and amplifying the HCV cDNA using the PCR method.

RNA was extracted from 100 μl of serum following treatment with proteinase K and sodium dodecylsulfate (SDS). The samples were extracted with phenol-chloroform, and the RNA precipitated with ethanol.

HCV cDNA from the J1 isolate was prepared by denaturing the precipitated RNA with 0.01M MeHgOH; after ten minutes at room temperature, 2-mercaptoethanol was added to sequester the mercury ions. Immediately, the mix for the first strand of cDNA synthesis was added, and incubation was continued for 1 hr at 37°C. The conditions for the synthesis of the anti-sense strand were the following: 50 mM Tris HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 500μM each deoxynucleotide triphosphate, 250 pmol specific antisense cDNA primer r25, 250 units MMLV reverse transcriptase. In order to synthesize the second strand (sense), the synthesis reaction components were added, and incubated for one hour at 14°C. The components for the second strand reaction were as follows: 14 mM Tris HCl, pH 8.3, 68 mM KCl, 7.5 mM ammonium sulfate, 3.5 mM $MgCl_2$, 2.8 mM dithiothreitol, 25 units DNA polymerase I, and one unit

RNase H. The reactions were terminated by heating the samples to 95°C for 10 minutes, followed by cooling on ice.

The HCV cDNA was amplified by two rounds of PCR. The first round was accomplished using 20 μl of the cDNA mix. The conditions for the PCR reaction were as follows: 10 mM Tris HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.002% gelating, 200 mM each of the deoxynucleotide triphosphates, and 2.5 units Amplitaq. The PCR thermal cycle was as follows: 94°C one minute, 50°C one minute, 72°C one minute, repeated 40 times followed by seven minutes at 72°C. The second round of PCR was accomplished using nested primers (i.e. primers which bound to an internal region of the first round of PCR amplified product) to increase the specificity of the PCR products. One percent of the first PCR reaction was amplified essentially as the first round, except that the primers were substituted, and the second step in the PCR reaction was at 60°C instead of 50°C. The primers used for the first round of PCR were ALX90 and r14. The primers used for the second round of PCR were r14 and p14.

The sequences of the primers for the synthesis of HCV cDNA and for the PCR method were the following.

r25

5′ ACC TTA CCC AAA TTG CGC GAC CTA 3′

ALX90

5′ CCA TGA ATC ACT CCC CTG TGA GGA ACT A 3′

r14

5′ GGG CCC CCAG CTA GGC CGA GA 3′

p14

5′ AAC TAC TGT CTT CAC GCA GAA AGC 3′

The PCR products were gel purified, the material which migrated as having about 615 bp was

isolated, and sequenced by a modification of the Sanger dideoxy chain termination method, using ^{32}P-ATP as label. In the modified method, the sequence replication was primed using P32 and R31 as primers; the double stranded DNA was melted for 3 minutes at 95°C prior to replication, and the synthesis of labeled dideoxy terminated polynucleotides was catalyzed by Bst polymerase (obtained from BioRad Corp.), according to the manufacturer's directions. The sequencing was performed using 500ng to 1 μg of PCR product per sequencing reaction.

The primers P32 (sense) and R31 (antisense) were derived from nucleotides -137 to -115 and from nucleotides 192 to 173, respectively, of the HCV1 sequence. The sequences of the primers are the following.

P32 primer

5′ AAC CCG CTC AAT GCC TGG AGA TT 3′

R31 primer

5′ GGC CGX CGA GCC TTG GGG AT 3′

where X = A or G

The sequence of the region in the J1 isolate which encompasses the 5′-untranslated region as well as a part of the region of the putative "Core" is shown in Figure 18. In the figure, amino acids encoded in the J1 sequence are shown above the nucleotide sequence. The sequence of the prototype HCV1 is shown below the J1 sequence; the dashes indicate sequence homology with J1. The differing amino acids encoded in the HCV1 sequence are shown below the HCV1 sequence.

An HCV cDNA fragment which is a representative of the 600 bp J1 sequence described above (TC 600bp) was cloned into pGEM3Z and maintained in host HB101; the HCV

cDNA fragment may be removed with SacI and XbaI. This material is on deposit with the ATCC.

Patent Microorganism Depository-deposited under Budapest Treaty terms.

Deposited Materials	Accession Number	Deposit Date
E. coli DH5/pS1-8791a	BP-2593	9/15/1989
(This clone contains 427 bp of the HS5 domain of J1)		
E. coli HB101/pU1-1216c	BP-2594	9/15/1989
(This clone contains 351 bp of the E/NS1 domains of J1)		
E. coli HB101/pU1-4652d	BP-2595	9/15/1989
(This clone contains 583 bp of the NS3 domain of J1)		
E.coli DH5α/pS1-713c	BP-2637	11/1/1989
(This clone contains 580 bp of the E domain of J1)		
E. coli DH5α/pS7-28c	BP-2638	11/1/1989
(This clone contains 552 bp of the C/E domain of J7)		
E. coli DH5α/ps1-1519	BP3081	8/30/90

The following vectors described in the Examples were deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville, Maryland 20852, and have been assigned the following Accession Numbers. The deposits were made under the terms of the Budapest Treaty.

Deposited Materials	Accession Number	Deposit Date
TC-600BP (in E. coli HB101/pGEM3Z)	68393	9/11/90
JH-400bp (in E. coli HB101/pGEM3Z)	68394	9/11/90
AW-300bp (in E. coli HB101/pGEM3Z)	68392	9/11/90
AW-770bp-N (in E. coli HB101/pM1E)	68395	9/11/90
AW-700bp-C or AW-700bp-N (in E. coli DH5α-F′/M13mp10)		

J1 5-1-1 (in
E. coli DH5α-F'/M13mp10)
HCV-J1 λ gt22 40884 9/6/90

These deposits are provided for the convenience of those skilled in the art. These deposits are neither an admission that such deposits are required to practice the present invention nor that equivalent embodiments are not within the skill of the art in view of the present disclosure. The public availability of these deposits is not a grant of a license to make, use or sell the deposited materials under this or any other patent. The nucleic acid sequences of the deposited materials are incorporated in to present disclosure by reference, and are controlling if in conflict with any sequences described herein.

While the present invention has been described by way specific examples for the benefit of those in the field, the scope of the invention is not limited as additional embodiments will be apparent to those of skill in the art from the present disclosure.

CLAIMS

1. A DNA molecule comprising a nucleotide sequence of at least 15 bp from an HCV isolate substantially homologous to an isolate selected from the group J1 and J7, wherein said nucleotide sequence is distinct from the nucleotide sequence of HCV isolate HCV1.

2. A DNA molecule comprising a nucleotide sequence of at least 15 bp encoding an amino acid sequence from the HCV isolate J1 or J7 wherein the J1 or J7 amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1.

3. A DNA molecule according to claim 2 wherein the J1 or J7 amino acid sequence comprises a substantially complete viral polypeptide.

4. A DNA molecule according to claim 2 wherein the J7 amino acid sequence is amino acid 1 to amino acid 115.

5. A DNA molecule according to claim 1 wherein the J1 amino acid sequence is from amino acid 116 to amino acid 350.

6. A DNA molecule according to claim 2 wherein the J1 amino acid sequence is from amino acid 351 to amino acid 651.

7. A DNA molecule according to claim 2 wherein the J1 amino acid sequence is from amino acid 1007 to amino acid 1650.

8. A DNA molecule according to claim 2 wherein the J1 amino acid sequence is from amino acid 2100 to the end of the coding sequence.

9. A purified polypeptide comprising an amino acid sequence from an HCV isolate substantially homologous to an isolate selected from the group consisting of J1 and J7 wherein the amino acid sequence is distinct from the sequence of the polypeptides encoded by the HCV isolate HCV1.

10. A purified polypeptide according to claim 9 wherein the J1 or J7 amino acid sequence comprises an epitope that is not immunologically cross-reactive with any HCV1 epitope.

11. A purified polypeptide according to claim 9 wherein the J7 amino acid sequence is from amino acid 1 to amino acid 115.

12. A purified polypeptide according to claim 9 wherein the J1 amino acid sequence is from amino acid 116 to amino acid 350.

13. A purified polypeptide according to claim 9 wherein the J1 amino acid sequence is from amino acid 351 to amino acid 651.

14. A purified polypeptide according to claim 9 wherein the J1 amino acid sequence is from amino acid 1007 to amino acid 1650.

15. A purified polypeptide according to claim 9 wherein the J1 amino acid sequence is from amino acid 2100 to the end of the coding sequence.

16. A polypeptide comprising an amino acid sequence from an HCV isolate selected from the group consisting of J1 and J7 wherein the J1 or J7 amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1 and the polypeptide is immobilized on a solid support.

17. An immunoassay for detecting the presence of anti-HCV antibodies in a test sample comprising:

(a) incubating the test sample under conditions that allow the formation of antigen-antibody complexes with an antigenic polypeptide comprising an amino acid sequence from an HCV isolate selected from the group consisting of J1 and J7, wherein the amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1; and (b) the J1 or J7 amino acid sequence is not immunologically cross-reactive with HCV1; and

(b) detecting an antigen-antibody complex comprising the antigenic polypeptide.

18. An immunoassay according to claim 17 wherein the J1 amino acid sequence is from a viral domain selected from the group consisting of amino acid sequence from amino acid 116 to amino acid 350, from amino acid 351 to amino acid 651, from amino acid 1007 to amino acid 1650, and from amino acid 2100 to the coding sequence.

19. An immunoassay according to claim 17 wherein the J1 amino acid sequence is from amino acid 1 to amino acid 115.

20. An immunoassay according to claim 17 wherein the test sample comprises human blood or a fraction thereof.

21. A composition comprising anti-HCV antibodies that bind an HCV epitope substantially free of antibodies that do not bind an HCV epitope, wherein:

(a) the HCV epitope comprises an amino acid sequence from an HCV isolate selected from the group consisting of J1 and J7;

(b) the J1 of J7 amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1; and

(c) the J1 or J7 amino acid sequence is not immunologically cross-reactive with HCV1.

22. A composition according to claim 21 wherein the anti-HCV antibodies are polyclonal.

23. A composition according to claim 21 wherein the anti-HCV antibodies are monoclonal.

24. An immunoassay for detecting the presence of an HCV polypeptide in a test sample comprising:

(a) incubating the test sample under conditions that allow the formation of antigen-antibody complexes with anti-HCV antibodies that bind an HCV epitope wherein:

(i) the HCV epitope comprises an amino acid sequence from an HCV isolate selected from the group consisting of J1 and J7;

(ii) the J1 or J7 amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1; and

(iii) the J1 or J7 amino acid sequence is not immunologically cross-reactive with HCV1; and

(b) detecting an antigen-antibody complex comprising the anti-HCV antibodies.

25. An immunoassay according to claim 24 wherein the J1 amino acid sequence is from a viral domain selected from the group consisting of amino acid sequence from amino acid 116 to amino acid 350, from amino acid 351 to amino acid 651, from amino acid 1007 to amino acid 1650, and from amino acid 2100 to the end of the coding sequence.

26. An immunoassay according to claim 24 wherein the J7 amino acid sequence is from amino acid 1 to amino acid 115.

27. A method of producing anti-HCV antibodies comprising administering to a mammal a polypeptide comprising an amino acid sequence from an HCV isolate selected from the group consisting of J1 and J7 wherein the J1 or J7 amino acid sequence is distinct from the amino acid sequence of HCV isolate HCV1 whereby the mammal produces anti-HCV antibodies.

28. A method of detecting HCV polynucleotides in a test sample comprising:

(a) providing a probe comprising the DNA molecule of claim 1;

(b) contacting the test sample and the probe under conditions that allow for the formation

of a polynucleotide duplex between the probe and its complement in the absence of substantial polynucleotide duplex formation between the probe and non-HCV polynucleotide sequences present in the test sample; and
(c) detecting any polynucleotide duplexes comprising the probe.

29. A method of producing a recombinant polypeptide comprising an HCV amino acid sequence, the method comprising:
(a) providing host cells transformed by a DNA construct comprising a control sequence for the host cell operably linked to a coding sequence for the host cell operably linked to a coding sequence encoding an amino acid sequence from an HCV isolate selected from the group comprised of J1 and J7 wherein the J1 or J7 amino is distinct from the amino acid sequence of HCV isolate HCV1;
(b) growing the host cells under conditions whereby the coding sequence is transcribed and translated into the recombinant polypeptide; and
(c) recovering the recombinant polypeptide.

30. A biological material derived from the group consisting of materials deposited under Accession Numbers BP-2593, BP-2594, BP-2595, BP-2637, BP-2638, BP-3081, ATCC No. 68392, ATCC No. 68393, ATCC No. 68394, ATCC No. 68395, and ATCC No. 408884.

```
J7        1     AGCCGAGTAGTGTTGGGTCGCGAAAGGCCTTGTGGT
discrepancy
clone
altered aa


J7       37     ACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGG


                                        Met Ser Thr Asn
J7       73     TCTCGTAGACCGTGCATC ATG AGC ACA AAT

                Pro Lys Pro Gln Arg Lys Thr Lys Arg
J7      103     CCT AAA CCC CAA AGA AAA ACC AAA CGT
                      T G
                      b l
                     --- Arg

                Asn Thr Asn Arg Arg Pro Gln Asp Val
J7      130     AAC ACC AAC CGT CGC CCA CAG GAC GTT
                                                C
                                                b
                                               ---

                Lys Phe Pro Gly Gly Gly Gln Ile Val
J7      157     AAG TTC CCG GGC GGT GGT CAG ATC GTC
                      T                         T
                      l                         b
                     Leu                       ---

                Gly Gly Val Tyr Leu Leu Pro Arg Arg
J7      184     GGT GGA GTT TAC TTG TTG CCG CGC AGG
                                           A
                                           b
                                          ---


                Gly Pro Arg Leu Gly Val Arg Ala Thr
J7      211     GGC CCC AGG TTG GGT GTG CGT GCG ACT
```

FIG. 1-1

```
                Arg Lys Thr Ser Glu Arg Ser Gln Pro
J7      238     AGG AAG ACT TCC GAG CGG TCG CAA CCT
                                        A
                                        b
                                        ---

                Arg Gly Arg Arg Gln Pro Ile Pro Lys
J7      265     CGT GGA AGG CGA CAA CCT ATC CCC AAG

                Ala Arg Arg Pro Glu Gly Arg Thr Trp
J7      292     GCT CGC CGG CCC GAG GGC AGG ACC TGG

                Ala Gln Pro Gly Tyr Pro Trp Pro Leu
J7      319     GCT CAG CCT GGG TAT CCT TGG CCC CTC

                Tyr Gly Asn Glu Gly Leu Gly Trp Ala
J7      346     TAT GGC AAT GAG GGC TTG GGG TGG GCA
                                    A
                                    b
                                    END

                Gly Trp Leu Leu Ser Pro Arg Gly Ser
J7      373     GGA TGG CTC CTG TCA CCC CGC GGC TCT

                Arg Pro Ser Trp Gly Pro Asn Asp Pro
J7      400     CGG CCT AGT TGG GGC CCC AAT GAC CCC
                                    T   C
                                    c   b
                                    ---
                                    Thr

                Arg Arg Arg Ser Arg Asn Leu Gly Lys
J7      427     CGG CGT AGG TCG CGT AAT TTG GGT AAG

                Val Ile Asp Thr Leu Thr Cys Gly Phe
J7      454     GTC ATC GAT ACC CTT ACA TGC GGC TTC
                                                C
                                                l
                                                Leu
```

FIG. 1-2

			Ala	Asp	Leu	Met	Gly	Tyr	Ile	Pro	Leu
J7		481	GCC	GAC	CTC	ATG	GGG	TAC	ATT	CCG	CTT
									C		C
									c		b
									---		---
			Val	Gly	Ala	Pro	Leu	Gly	Gly	Ala	Ala
J7		508	GTC	GGC	GCC	CCC	TTA	GGG	GGC	GCT	GCC
			Arg	Ala	Leu	Ala	His	Gly			
J7		535	AGG	GCC	CTG	GCA	CAT	GGT			

FIG. 1-3

```
                  Pro Leu Val Gly Ala Pro Leu Gly Gly
J1      1     T   CCG CTC GTC GGC GCC CCC TTA GGG GGC
discrepancy                                   C
clone                                         d
altered aa                                    Ser

                  Ala Ala Arg Ala Leu Ala His Gly Val
  29              GCT GCC AGG GCC CTG GCA CAT GGT GTC

                  Arg Val Leu Glu Asp Gly Val Asn Tyr
  56              CGG GTT CTG GAG GAC GGC GTG AAC TAT

                  Ala Thr Gly Asn Leu Pro Gly Cys Ser
  83              GCA ACA GGG AAT TTG CCC GGT TGC TCT

                  Phe Ser Ile Phe Leu Leu Ala Leu Leu
 110              TTC TCT ATC TTC CTC TTG GCT CTG CTG
                                       A      T
                                       g      d
                                      ---    ---

                  Ser Cys Leu Thr Ile Pro Ala Ser Ala
 137              TCC TGT TTG ACC ATC CCA GCT TCC GCT

                  Tyr Glu Val Arg Asn Val Ser Gly Ile
 164              TAT GAA GTG CGC AAC GTG TCC GGG ATA

                  Tyr His Val Thr Asn Asp Cys Ser Asn
 191              TAC CAT GTC ACA AAC GAC TGC TCC AAC
                    T
                    d
                   ---

                  Ser Ser Ile Val Tyr Glu Ala Ala Asp
 218              TCA AGC ATT GTG TAT GAG GCG GCG GAC
```

FIG. 2-1

```
                Val Ile Met His Ala Pro Gly Cys Val
245             GTG ATC ATG CAT GCC CCC GGG TGC GTG

                Pro Cys Val Arg Glu Asn Asn Ser Ser
272             CCC TGC GTT CGG GAG AAC AAT TCC TCC
                                          C
                                          d
                                          ---

                Arg Cys Trp Val Ala Leu Thr Pro Thr
299             CGT TGC TGG GTA GCG CTC ACT CCC ACG

                Leu Ala Ala Arg Asn Ala Ser Val Pro
326             CTC GCG GCC AGG AAT GCC AGC GTC CCC

                Thr Thr Thr Leu Arg Arg His Val Asp
353             ACT ACG ACA TTA CGA CGC CAC GTC GAC
                           G
                           d
                          ---

                Leu Leu Val Gly Thr Ala Ala Phe Cys
380             TTG CTC GTT GGG ACG GCT GCT TTC TGC

                Ser Ala Met Tyr Val Gly Asp Leu Cys
407             TCC GCT ATG TAC GTG GGG GAT CTC TGC

                Gly Ser Val Phe Leu Ile Ser Gln Leu
434             GGA TCT GTT TTC CTC ATC TCC CAG CTG
                                    T
                                    d
                                   ---

                Phe Thr Phe Ser Pro Arg Arg His Glu
461             TTC ACC TTC TCG CCT CGC CGG CAT GAG
```

FIG. 2-2

```
                Thr Val Gln Asp Cys Asn Cys Ser Ile
488             ACA GTA CAG GAC TGC AAC TGC TCA ATC

                Tyr Pro Gly His Val Ser Gly His Arg
515             TAT CCC GGC CAC GTA TCA GGC CAT CGC
                                                  T
                                                  C
                                                  ---

                Met Ala Trp Asp Met Met Met Asn Trp
542             ATG GCT TGG GAT ATG ATG ATG AAC TGG

                Ser Pro Thr Ala
569             TCG CCC ACG GCA
```

FIG. 2-3

```
                                    Asn Trp Ser Pro Thr
      J1                1           AAC TGG TCG CCC ACG
      discrepancy
      clone
      altered aa

                        Ala Ala Leu Val Val Ser Gln Leu Leu
J1    16                GCA GCC TTA GTG GTG TCG CAG TTA CTC
                        !!!

                        Arg Ile Pro Gln Ala Val Met Asp Met
J1    43                CGG ATC CCA CAA GCT GTC ATG GAC ATG

                        Val Ala Gly Ala His Trp Gly Val Leu
J1    70                GTG GCG GGG GCC CAC TGG GGA GTC CTA
                                                        G
                                                        i
                                                        ---

                        Ala Gly Leu Ala Tyr Tyr Ser Met Val
J1    97                GCG GGC CTT GCC TAC TAT TCC ATG GTG
                                                        A
                                                        i
                                                        ---

                        Gly Asn Trp Ala Lys Val Leu Ile Val
J1    124               GGG AAC TGG GCT AAG GTT TTG ATT GTG

                        Met Leu Leu Phe Ala Gly Val Asp Gly
J1    151               ATG CTA CTC TTT GCC GGC GTT GAC GGG

                        His Thr Arg Val Thr Gly Gly Val Gln
J1    178               CAT ACC CGC GTG ACG GGG GGG GTG CAA
                        AG                    A
                        gg                    i
                        Ser                   ---
```

FIG. 3-1

```
                    Gly His Val Thr Ser Thr Leu Thr Ser
J1    205           GGC CAC GTC ACC TCT ACA CTC ACG TCC
                        T                 G
                        c                 i
                        ---               Ala

                    Leu Phe Arg Pro Gly Ala Ser Gln Lys
J1    232           CTC TTT AGA CCT GGG GCG TCC CAG AAA

                    Ile Gln Leu Val Asn Thr Asn Gly Ser
J1    259           ATT CAG CTT GTA AAC ACC AAT GGC AGT
                          TC  T
                          ii  i
                          Ser Leu

                    Trp His Ile Asn Arg Thr Ala Leu Asn
J1    286           TGG CAT ATC AAC AGG ACT GCC CTG AAC
                                                    T
                                                    g
                                                    ---

                    Cys Asn Asp Ser Leu Gln Thr Gly Phe
J1    313           TGC AAT GAC TCC CTC CAA ACT GGG TTC

                    Leu Ala Ala Leu
J1    340           CTT GCC GCG CTG
```

FIG. 3-2

```
                                                    Ser
                J1          1                   C TCA
                discrepancy
                clone
                altered aa


                    Val Ile Asp Cys Asn Thr Cys Val Thr
J1      5           GTG ATC GAC TGT AAC ACA TGT GTC ACT


                    Gln Thr Val Asp Phe Ser Leu Asp Pro
J1      32          CAG ACG GTC GAT TTC AGC TTG GAT CCC


                    Thr Phe Thr Ile Glu Thr Thr Thr Val
J1      59          ACC TTC ACC ATC GAG ACG ACG ACC GTG
                    G
                    c
                    Ala


                    Pro Gln Asp Ala Val Ser Arg Thr Gln
J1      86          CCC CAA GAT GCG GTT TCG CGC ACG CAG


                    Arg Arg Gly Arg Thr Gly Arg Gly Arg
J1      113         CGG CGA GGT AGG ACT GGC AGG GGC AGG


                    Arg Gly Ile Tyr Arg Phe Val Thr Pro
J1      140         AGA GGC ATC TAT AGG TTT GTG ACT CCA


                    Gly Glu Arg Pro Ser Ala Met Phe Asp
J1      167         GGA GAA CGG CCC TCG GCG ATG TTC GAT


                    Ser Ser Val Leu Cys Glu Cys Tyr Asp
J1      194         TCT TCG GTC CTA TGT GAG TGT TAT GAC


                    Ala Gly Cys Ala Trp Tyr Glu Leu Thr
J1      221         GCG GGC TGT GCT TGG TAT GAG CTC ACG
                          A
                          e
                    Gly(=)
```

FIG. 4-1

```
                Pro Ala Glu Thr Ser Val Arg Leu Arg
J1      248     CCC GCT GAG ACC TCG GTT AGG TTG CGG

                Ala Tyr Leu Asn Thr Pro Gly Leu Pro
J1      275     GCT TAC CTA AAT ACA CCA GGG TTG CCC

                Val Cys Gln Asp His Leu Glu Phe Trp
J1      302     GTC TGC CAG GAC CAT CTG GAG TTC TGG

                Glu Ser Val Phe Thr Gly Leu Thr His
J1      329     GAG AGC GTC TTC ACA GGC CTC ACC CAC

                Ile Asp Ala His Phe Leu Ser Gln Thr
J1      356     ATA GAC GCC CAC TTC TTG TCC CAG ACT

                Lys Gln Ala Gly Asp Asn Phe Pro Tyr
J1      383     AAG CAG GCA GGA GAC AAC TTC CCC TAC

                Leu Val Ala Tyr Gln Ala Thr Val Cys
J1      410     CTG GTA GCA TAC CAA GCC ACA GTG TGC

                Ala Arg Ala Lys Ala Pro Pro Pro Ser
J1      437     GCC AGG GCT AAG GCT CCA CCT CCA TCG
                                    C
                                    e
                                Ala(=)

                Trp Asp Gln Met Trp Lys Cys Leu Ile
J1      464     TGG GAT CAA ATG TGG AAG TGT CTC ATA

                Arg Leu Lys Pro Thr Leu His Gly Pro
J1      491     CGG CTA AAG CCT ACG CTG CAC GGG CCA
                                              G
                                              e
                                            Ala
```

FIG. 4-2

```
                Thr Pro Leu Leu Tyr Arg Leu Gly Ala
J1      518     ACG CCC CTG CTG TAT AGG CTA GGA GCC
                                      A
                                      e
                                   Arg(=)

                Val Gln Asn Glu Val Thr Leu Thr His
J1      545     GTC CAG AAT GAG GTC ACC CTC ACA CAC

                Pro Ile Thr Lys
J1      572     CCT ATA ACC AAA
```

FIG. 4-3

```
                                                    Leu Thr
J1                  1                           C CTC ACC
discrepancy
clone
altered aa

                    Arg Asp Pro Thr Val Pro Leu Ala Arg
J1          8       CGT GAC CCC ACC GTC CCC CTT GCG CGG

                    Ala Ala Trp Glu Thr Ala Arg His Thr
J1          35      GCT GCG TGG GAG ACA GCT AGA CAC ACT
                                                      C
                                                      g
                                                    Thr(=)

                    Pro Val Asn Ser Trp Leu Gly Asn Ile
J1          62      CCA GTC AAC TCC TGG CTA GGC AAC ATC

                    Ile Met Tyr Ala Pro Thr Leu Trp Ala
J1          89      ATC ATG TAT GCG CCC ACT TTG TGG GCA
                      T
                      g
                    Ile(=)

                    Arg Met Ile Leu Met Thr His Phe Phe
J1          116     AGG ATG ATT CTG ATG ACT CAC TTC TTC

                    Ser Ile Leu Leu Ala Gln Glu Gln Leu
J1          143     TCC ATC CTT CTA GCC CAG GAG CAA CTT

                    Glu Lys Ala Leu Asp Cys Gln Ile Tyr
J1          170     GAA AAA GCC CTG GAT TGT CAA ATC TAC

                    Gly Ala Cys Tyr Ser Ile Glu Pro Leu
J1          197     GGG GCC TGT TAC TCC ATT GAG CCA CTT
```

FIG. 5-1

```
                    Asp Leu Pro Gln Ile Ile Glu Arg Leu
J1          224     GAC CTA CCT CAG ATC ATT GAA CGA CTC

                    His Gly Leu Ser Ala Phe Ser Leu His
J1          251     CAT GGT CTT AGC GCA TTT TCA CTC CAT

                    Ser Tyr Ser Pro Gly Glu Ile Asn Arg
J1          278     AGT TAC TCT CCA GGT GAG ATC AAT AGG

                    Val Ala Ser Cys Leu Arg Lys Leu Gly
J1          305     GTG GCT TCA TGC CTC AGG AAG CTT GGG

                    Val Pro Pro Leu Arg Val Trp Arg His
J1          332     GTA CCA CCC TTG CGA GTC TGG AGA CAT

                    Arg Ala Arg Ser Val Arg Ala Lys Leu
J1          359     CGG GCC AGA AGT GTC CGC GCT AAG CTA

                    Leu Ser Gln Gly Gly Arg Ala Ala Thr
J1          386     CTG TCC CAA GGG GGG AGG GCC GCC ACT
                                G
                                g
                            Gln(=)

                    Lys Gly Lys Tyr Leu
J1          413     TGT GGC AAG TAC CTC
```

FIG. 5-2

```
J7      1    AGCCGAGTAGTGTTGGGTCGCGAAAGGCCTTGTGGT
HCV1

J7     37    ACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGG
HCV1

                                    Met  Ser  Thr  Asn
J7     73    TCTCGTAGACCGTGCATC   ATG  AGC  ACA  AAT
HCV1                           C                 G

                Pro  Lys  Pro  Gln  Arg  Lys  Thr  Lys  Arg
J7    103       CCT  AAA  CCC  CAA  AGA  AAA  ACC  AAA  CGT
HCV1                     T         A              A
                                   Lys           Asn
                                                 ***

                Asn  Thr  Asn  Arg  Arg  Pro  Gln  Asp  Val
J7    130       AAC  ACC  AAC  CGT  CGC  CCA  CAG  GAC  GTT
HCV1                                                    C

                Lys  Phe  Pro  Gly  Gly  Gly  Gln  Ile  Val
J7    157       AAG  TTC  CCG  GGC  GGT  GGT  CAG  ATC  GTC
HCV1                          T    C                    T

                Gly  Gly  Val  Tyr  Leu  Leu  Pro  Arg  Arg
J7    184       GGT  GGA  GTT  TAC  TTG  TTG  CCG  CGC  AGG
HCV1

                Gly  Pro  Arg  Leu  Gly  Val  Arg  Ala  Thr
J7    211       GGC  CCC  AGG  TTG  GGT  GTG  CGT  GCG  ACT
HCV1                T    A                        C         G

                Arg  Lys  Thr  Ser  Glu  Arg  Ser  Gln  Pro
J7    238       AGG  AAG  ACT  TCC  GAG  CGG  TCG  CAA  CCT
HCV1              A
```

FIG. 6-1

		Arg	Gly	Arg	Arg	Gln	Pro	Ile	Pro	Lys
J7	265	CGT	GGA	AGG	CGA	CAA	CCT	ATC	CCC	AAG
HCV1		A	T	A	T	G				
		Ala	Arg	Arg	Pro	Glu	Gly	Arg	Thr	Trp
J7	292	GCT	CGC	CGG	CCC	GAG	GGC	AGG	ACC	TGG
HCV1			T							
		Ala	Gln	Pro	Gly	Tyr	Pro	Trp	Pro	Leu
J7	319	GCT	CAG	CCT	GGG	TAT	CCT	TGG	CCC	CTC
HCV1				C		C				
		Tyr	Gly	Asn	Glu	Gly	Leu	Gly	Trp	Ala
J7	346	TAT	GGC	AAT	GAG	GGC	TTG	GGG	TGG	GCA
HCV1							GC			G
							Cys			
							***			
		Gly	Trp	Leu	Leu	Ser	Pro	Arg	Gly	Ser
J7	373	GGA	TGG	CTC	CTG	TCA	CCC	CGC	GGC	TCT
HCV1						T		T		
		Arg	Pro	Ser	Trp	Gly	Pro	Asn	Asp	Pro
J7	400	CGG	CCT	AGT	TGG	GGC	CCC	AAT	GAC	CCC
HCV1				C				CA		
								Thr		
								***		
		Arg	Arg	Arg	Ser	Arg	Asn	Leu	Gly	Lys
J7	427	CGG	CGT	AGG	TCG	CGT	AAT	TTG	GGT	AAG
HCV1						C				
		Val	Ile	Asp	Thr	Leu	Thr	Cys	Gly	Phe
J7	454	GTC	ATC	GAT	ACC	CTT	ACA	TGC	GGC	TTC
HCV1							G			

FIG. 6-2

			Ala	Asp	Leu	Met	Gly	Tyr	Ile	Pro	Leu
J7	481		GCC	GAC	CTC	ATG	GGG	TAC	ATT	CCG	CTT
HCV1									A		C
			Val	Gly	Ala	Pro	Leu	Gly	Gly	Ala	Ala
J7	508		GTC	GGC	GCC	CCC	TTA	GGG	GGC	GCT	GCC
HCV1						T	C	T	A		
			Arg	Ala	Leu	Ala	His	Gly			
J7	535		AGG	GCC	CTG	GCA	CAT	GGT			
HCV1						G		C			

FIG. 6-3

			Pro	Leu	Val	Gly	Ala	Pro	Leu	Gly	Gly
J1	1	T	CCG	CTC	GTC	GGC	GCC	CCC	TTA	GGG	GGC
		A						T	C T	A	
			Ala	Ala	Arg	Ala	Leu	Ala	His	Gly	Val
J1	29		GCT	GCC	AGG	GCC	CTG	GCA	CAT	GGT	GTC
								G		C	
			Arg	Val	Leu	Glu	Asp	Gly	Val	Asn	Tyr
J1	56		CGG	GTT	CTG	GAG	GAC	GGC	GTG	AAC	TAT
						A					
			Ala	Thr	Gly	Asn	Leu	Pro	Gly	Cys	Ser
J1	83		GCA	ACA	GGG	AAT	TTG	CCC	GGT	TGC	TCT
						C	C T	T			
			Phe	Ser	Ile	Phe	Leu	Leu	Ala	Leu	Leu
J1	110		TTC	TCT	ATC	TTC	CTC	TTG	GCT	CTG	CTG
							T	C	C		C
			Ser	Cys	Leu	Thr	Ile	Pro	Ala	Ser	Ala
J1	137		TCC	TGT	TTG	ACC	ATC	CCA	GCT	TCC	GCT
			T	C		T	G	C		G	C
							Val				
			Tyr	Glu	Val	Arg	Asn	Val	Ser	Gly	Ile
J1	164		TAT	GAA	GTG	CGC	AAC	GTG	TCC	GGG	ATA
			C	C				TCC	A G		C T
				Gln				Ser	Thr		Leu
				***				***			
			Tyr	His	Val	Thr	Asn	Asp	Cys	Ser	Asn
J1	191		TAC	CAT	GTC	ACA	AAC	GAC	TGC	TCC	AAC
				C		C	T	T		C T	
										Pro	

FIG. 7-1

		Ser	Ser	Ile	Val	Tyr	Glu	Ala	Ala	Asp
J1	218	TCA	AGC	ATT	GTG	TAT	GAG	GCG	GCG	GAC
		G		T		C			C	T
		Val	Ile	Met	His	Ala	Pro	Gly	Cys	Val
J1	245	GTG	ATC	ATG	CAT	GCC	CCC	GGG	TGC	GTG
		CC		C	C	A T	G			C
		Ala		Leu		Thr				
		Pro	Cys	Val	Arg	Glu	Asn	Asn	Ser	Ser
J1	272	CCC	TGC	GTT	CGG	GAG	AAC	AAT	TCC	TCC
		T			T		GG	C	G	G
							Gly		Ala	
							***			
		Arg	Cys	Trp	Val	Ala	Leu	Thr	Pro	Thr
J1	299	CGT	TGC	TGG	GTA	GCG	CTC	ACT	CCC	ACG
		A G	T		G		A G	C	T	
							Met			
		Leu	Ala	Ala	Arg	Asn	Ala	Ser	Val	Pro
J1	326	CTC	GCG	GCC	AGG	AAT	GCC	AGC	GTC	CCC
		G G	C	A		G	G	AA	C	
		Val		Thr		Asp	Gly	Lys	Leu	
						***		***		
		Thr	Thr	Thr	Leu	Arg	Arg	His	Val	Asp
J1	353	ACT	ACG	ACA	TTA	CGA	CGC	CAC	GTC	GAC
		G G		CAG	C T		T		A	T
		Ala		Gln					Ile	
				***						
		Leu	Leu	Val	Gly	Thr	Ala	Ala	Phe	Cys
J1	380	TTG	CTC	GTT	GGG	ACG	GCT	GCT	TTC	TGC
		C	T	C		GC	C	A C	C	T
						Ser		Thr	Leu	

FIG. 7-2

			Ser	Ala	Met	Tyr	Val	Gly	Asp	Leu	Cys
J1		407	TCC	GCT	ATG	TAC	GTG	GGG	GAT	CTC	TGC
			G	C	C C				C	A	
					Leu						
			Gly	Ser	Val	Phe	Leu	Ile	Ser	Gln	Leu
J1		434	GGA	TCT	GTT	TTC	CTC	ATC	TCC	CAG	CTG
			G		C	T	T	G	GG	A	
								Val	Gly		
			Phe	Thr	Phe	Ser	Pro	Arg	Arg	His	Glu
J1		461	TTC	ACC	TTC	TCG	CCT	CGC	CGG	CAT	GAG
						T	C	A G	C	C	TG
											Trp
											***
			Thr	Val	Gln	Asp	Cys	Asn	Cys	Ser	Ile
J1		488	ACA	GTA	CAG	GAC	TGC	AAC	TGC	TCA	ATC
			G	ACG	A	GT		T		T	
				Thr		Gly					
				***		***					
			Tyr	Pro	Gly	His	Val	Ser	Gly	His	Arg
J1		515	TAT	CCC	GGC	CAC	GTA	TCA	GGC	CAT	CGC
						T	A	A G	T	C	
							Ile	Thr			
			Met	Ala	Trp	Asp	Met	Met	Met	Asn	Trp
J1		542	ATG	GCT	TGG	GAT	ATG	ATG	ATG	AAC	TGG
				A							
			Ser	Pro	Thr	Ala					
J1		569	TCG	CCC	ACG	GCA					
			C	T		A G					
						Thr					

FIG. 7-3

```
                                  Asn Trp Ser Pro Thr Ala
          J1      1               AAC TGG TCG CCC ACG GCA
        HCV1                              C   T       A G
                                                      Thr

                  Ala Leu Val Val Ser Gln Leu Leu Arg
          J1  19  GCC TTA GTG GTG TCG CAG TTA CTC CGG
        HCV1        G   G   A A   G T       C G
                                Met Ala

                  Ile Pro Gln Ala Val Met Asp Met Val
          J1  46  ATC CCA CAA GCT GTC ATG GAC ATG GTG
        HCV1                    C A   T           A C
                                  Ile Leu           Ile

                  Ala Gly Ala His Trp Gly Val Leu Ala
          J1  73  GCG GGG GCC CAC TGG GGA GTC CTA GCG
        HCV1        T   T   T                   G
```

```
                  Gly Leu Ala Tyr Tyr Ser Met Val Gly
          J1 100  GGC CTT GCC TAC TAT TCC ATG GTG GGG
        HCV1          A A   G   T  TC
                      Ile           Phe

                  Asn Trp Ala Lys Val Leu Ile Val Met
          J1 127  AAC TGG GCT AAG GTT TTG ATT GTG ATG
        HCV1              G       C C   G A     C
                                        Val     Leu

                  Leu Leu Phe Ala Gly Val Asp Gly His
          J1 154  CTA CTC TTT GCC GGC GTT GAC GGG CAT
        HCV1        G   A                 C     C  G A
                                              Ala Glu
                                                  ***

                  Thr Arg Val Thr Gly Gly Val Gln Gly
          J1 181  ACC CGC GTG ACG GGG GGG GTG CAA GGC
        HCV1        A   C   C       A AGT GCC
                  His               Ser Ala
                                    *** ***
```

FIG. 8-1

```
                    His Val Thr Ser Thr Leu Thr Ser Leu
      J1  208       CAC GTC ACC TCT ACA CTC ACG TCC CTC
HCV1                    ACT GTG     GG  T T GTT AG
                        Thr Val     Gly Phe Val
                        *** ***             ***

                    Phe Arg Pro Gly Ala Ser Gln Lys Ile
J1  235             TTT AGA CCT GGG GCG TCC CAG AAA ATT
HCV1                C C GC    A   C   C AAG     C G C
                    Leu Ala                 Lys     Asn Val
                        ***                 ***     ***

                    Gln Leu Val Asn Thr Asn Gly Ser Trp
J1  262             CAG CTT GTA AAC ACC AAT GGC AGT TGG
HCV1                    G A C           C
                        Ile

                    His Ile Asn Arg Thr Ala Leu Asn Cys
      J1  289       CAT ATC AAC AGG ACT GCC CTG AAC TGC
HCV1                  C C     T   C   G
                        Leu     Ser
                                ***

                    Asn Asp Ser Leu Gln Thr Gly Phe Leu
      J1  316       AAT GAC TCC CTC CAA ACT GGG TTC CTT
HCV1                    T AG        A C   C   C  GG T G
                                    Asn       Trp

                    Ala Ala Leu
J1  343             GCC GCG CTG
HCV1                  A  G    T
                        Gly
```

FIG. 8-2

```
                                                    Ser Val Ile
J1       1                                        C TCA GTG ATC
HCV1                    ggctataccggcgacttcga        G       A

                  Asp Cys Asn Thr Cys Val Thr Gln Thr
J1       11       GAC TGT AAC ACA TGT GTC ACT CAG ACG
HCV1                   C   T   G               C       A

                  Val Asp Phe Ser Leu Asp Pro Thr Phe
J1       38       GTC GAT TTC AGC TTG GAT CCC ACC TTC
HCV1                                C T   C   T

                  Thr Ile Glu Thr Thr Thr Val Pro Gln
J1       65       ACC ATC GAG ACG ACG ACC GTG CCC CAA
HCV1                   T       A  TC   G C C       G
                                  Ile

                  Asp Ala Val Ser Arg Thr Gln Arg Arg
J1       92       GAT GCG GTT TCG CGC ACG CAG CGG CGA
HCV1                   T   C   C       T   A   T   G

                  Gly Arg Thr Gly Arg Gly Arg Arg Gly
J1       119      GGT AGG ACT GGC AGG GGC AGG AGA GGC
HCV1               C                   G   A   CC
                                           Lys Pro

                  Ile Tyr Arg Phe Val Thr Pro Gly Glu
J1       146      ATC TAT AGG TTT GTG ACT CCA GGA GAA
HCV1                   C   A           G A   G   G   G
                                       Ala

                  Arg Pro Ser Ala Met Phe Asp Ser Ser
J1       173      CGG CCC TCG GCG ATG TTC GAT TCT TCG
HCV1               C       C  GC              C   G   C
                              Gly
```

FIG. 9-1

		Val	Leu	Cys	Glu	Cys	Tyr	Asp	Ala	Gly
J1	200	GTC	CTA	TGT	GAG	TGT	TAT	GAC	GCG	GGC
HCV1			C			C			A	
		Cys	Ala	Trp	Tyr	Glu	Leu	Thr	Pro	Ala
J1	227	TGT	GCT	TGG	TAT	GAG	CTC	ACG	CCC	GCT
HCV1										C
		Glu	Thr	Ser	Val	Arg	Leu	Arg	Ala	Tyr
J1	254	GAG	ACC	TCG	GTT	AGG	TTG	CGG	GCT	TAC
HCV1			T	A A			C A	A	G	
				Thr						
		Leu	Asn	Thr	Pro	Gly	Leu	Pro	Val	Cys
J1	281	CTA	AAT	ACA	CCA	GGG	TTG	CCC	GTC	TGC
HCV1		A G	C	C	G		C T		G	
		Met								
		Gln	Asp	His	Leu	Glu	Phe	Trp	Glu	Ser
J1	308	CAG	GAC	CAT	CTG	GAG	TTC	TGG	GAG	AGC
HCV1					T	A	T			G
										Gly
		Val	Phe	Thr	Gly	Leu	Thr	His	Ile	Asp
J1	335	GTC	TTC	ACA	GGC	CTC	ACC	CAC	ATA	GAC
HCV1			T				T	T		T
		Ala	His	Phe	Leu	Ser	Gln	Thr	Lys	Gln
J1	362	GCC	CAC	TTC	TTG	TCC	CAG	ACT	AAG	CAG
HCV1				T	C A			A		
		Ala	Gly	Asp	Asn	Phe	Pro	Tyr	Leu	Val
J1	389	GCA	GGA	GAC	AAC	TTC	CCC	TAC	CTG	GTA
HCV1		AGT	G	G		C T	T			
—		Ser		Glu		Leu				

FIG. 9-2

```
                    Ala Tyr Gln Ala Thr Val Cys Ala Arg
J1          416     GCA TAC CAA GCC ACA GTG TGC GCC AGG
HCV1                  G                 C           T

                    Ala Lys Ala Pro Pro Pro Ser Trp Asp
J1          443     GCT AAG GCT CCA CCT CCA TCG TGG GAT
HCV1                    C A   C   T   C                 C
                        Gln

                    Gln Met Trp Lys Cys Leu Ile Arg Leu
J1          470     CAA ATG TGG AAG TGT CTC ATA CGG CTA
HCV1                  G                 T G   T   C   C

                    Lys Pro Thr Leu His Gly Pro Thr Pro
J1          497     AAG CCT ACG CTG CAC GGG CCA ACG CCC
HCV1                    C   C   C   T           A

                    Leu Leu Tyr Arg Leu Gly Ala Val Gln
J1          524     CTG CTG TAT AGG CTA GGA GCC GTC CAG
HCV1                    A   C   A   G   C   T   T

                    Asn Glu Val Thr Leu Thr His Pro Ile
J1          551     AAT GAG GTC ACC CTC ACA CAC CCT ATA
HCV1                  A A         G   G       A G C
                        Ile                     Val

                    Thr Lys
J1          578     ACC AAA
HCV1                        tacatcatgacatgcatgtc
```

FIG. 9-3

```
                                                  Leu Thr
                                  J1    1         C CTC ACC
                                 HCV1

                  Arg Asp Pro Thr Val Pro Leu Ala Arg
  J1    8         CGT GAC CCC ACC GTC CCC CTT GCG CGG
 HCV1                    T   A AC       C      A A
                                 Thr
                                 ***

                  Ala Ala Trp Glu Thr Ala Arg His Thr
  J1    35        GCT GCG TGG GAG ACA GCT AGA CAC ACT
 HCV1                                 A

                  Pro Val Asn Ser Trp Leu Gly Asn Ile
  J1    62        CCA GTC AAC TCC TGG CTA GGC AAC ATC
 HCV1                    T                       A

                  Ile Met Tyr Ala Pro Thr Leu Trp Ala
  J1    89        ATC ATG TAT GCG CCC ACT TTG TGG GCA
 HCV1                    T   C       A C         G
                         Phe

                  Arg Met Ile Leu Met Thr His Phe Phe
  J1    116       AGG ATG ATT CTG ATG ACT CAC TTC TTC
 HCV1                    A           C   T       T

                  Ser Ile Leu Leu Ala Gln Glu Gln Leu
  J1    143       TCC ATC CTT CTA GCC CAG GAG CAA CTT
 HCV1             AG  G       A      AG   C   G
                      Val     Ile     Arg Asp
                                      ***

                  Glu Lys Ala Leu Asp Cys Gln Ile Tyr
  J1    170       GAA AAA GCC CTG GAT TGT CAA ATC TAC
 HCV1                C G     C       C G G
                     Gln             Glu
                     ***             ***
```

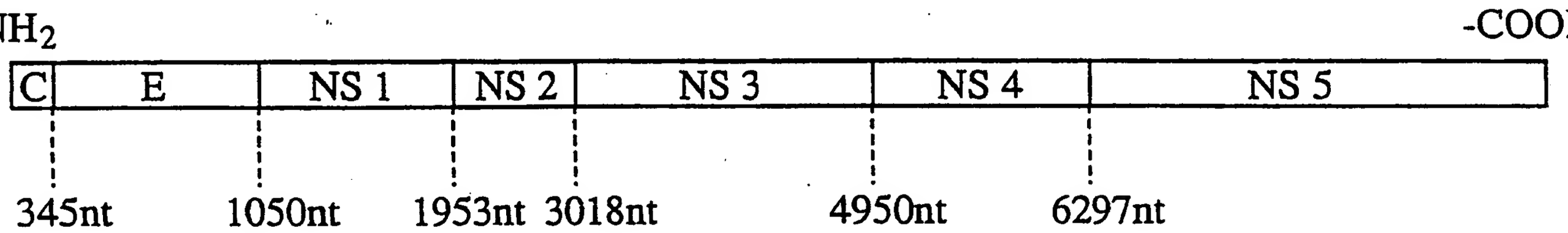
FIG. 10-1

		Gly	Ala	Cys	Tyr	Ser	Ile	Glu	Pro	Leu
J1	197	GGG	GCC	TGT	TAC	TCC	ATT	GAG	CCA	CTT
HCV1				C			A	A		
		Asp	Leu	Pro	Gln	Ile	Ile	Glu	Arg	Leu
J1	224	GAC	CTA	CCT	CAG	ATC	ATT	GAA	CGA	CTC
HCV1		T			CA			C	A	
					Pro			Gln		
					***			***		
		His	Gly	Leu	Ser	Ala	Phe	Ser	Leu	His
J1	251	CAT	GGT	CTT	AGC	GCA	TTT	TCA	CTC	CAT
HCV1			C	C						C
		Ser	Tyr	Ser	Pro	Gly	Glu	Ile	Asn	Arg
J1	278	AGT	TAC	TCT	CCA	GGT	GAG	ATC	AAT	AGG
HCV1							A	T		
		Val	Ala	Ser	Cys	Leu	Arg	Lys	Leu	Gly
J1	305	GTG	GCT	TCA	TGC	CTC	AGG	AAG	CTT	GGG
HCV1			C	G			A	A		
				Ala						
		Val	Pro	Pro	Leu	Arg	Val	Trp	Arg	His
J1	332	GTA	CCA	CCC	TTG	CGA	GTC	TGG	AGA	CAT
HCV1			G				CT			C
							Ala			
							***			
		Arg	Ala	Arg	Ser	Val	Arg	Ala	Lys	Leu
J1	359	CGG	GCC	AGA	AGT	GTC	CGC	GCT	AAG	CTA
HCV1				C G	C				G	T
									Arg	
		Leu	Ser	Gln	Gly	Gly	Arg	Ala	Ala	Thr
J1	386	CTG	TCC	CAA	GGG	GGG	AGG	GCC	GCC	ACT
HCV1			G	AG	A	C		T		TA
			Ala	Arg						Ile
				***						***

FIG. 10-2

		Lys	Gly	Lys	Tyr	Leu
J1	413	TGT	GGC	AAG	TAC	CTC
HCV1						

FIG. 10-3



The nucleotide numbers are approximate

FIG. 11

HCV-1

```
-267  GCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGG
      CGCAGATCGGTACCGCAATCATACTCACAGCACGTCGGAGGTCC

-223  ACCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGA
      TGGGGGGGAGGGCCCTCTCGGTATCACCAGACGCCTTGGCCACT

-179  GTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATCAA
      CATGTGGCCTTAACGGTCCTGCTGGCCCAGGAAAGAACCTAGTT

-135  CCCGCTCAATGCCTGGAGATTTGGGCGTGCCCCCGCAAGACTGC
      GGGCGAGTTACGGACCTCTAAACCCGCACGGGGGCGTTCTGACG

 -91  TAGCCGAGTAGTGTTGGGTCGCGAAAGGCCTTGTGGTACTGCCT
      ATCGGCTCATCACAACCCAGCGCTTTCCGGAACACCATGACGGA

 -47  GATAGGGTGCTTGCGAGTGCCCCGGGAGGTCTCGTAGACCGTGC
      CTATCCCACGAACGCTCACGGGGCCCTCCAGAGCATCTGGCACG

  -3  ACC -1
      TGG

          Met Ser Thr Asn Pro Lys Pro Gln Lys Lys Asn
   1  ATG AGC ACG AAT CCT AAA CCT CAA AAA AAA AAC
      TAC TCG TGC TTA GGA TTT GGA GTT TTT TTT TTG

          Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp Val
  34  AAA CGT AAC ACC AAC CGT CGC CCA CAG GAC GTC
      TTT GCA TTG TGG TTG GCA GCG GGT GTC CTG CAG

          Lys Phe Pro Gly Gly Gly Gln Ile Val Gly Gly
  67  AAG TTC CCG GGT GGC GGT CAG ATC GTT GGT GGA
      TTC AAG GGC CCA CCG CCA GTC TAG CAA CCA CCT
```

FIG. 12-1

```
      Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu
100   GTT TAC TTG TTG CCG CGC AGG GGC CCT AGA TTG
      CAA ATG AAC AAC GGC GCG TCC CCG GGA TCT AAC

      Gly Val Arg Ala Thr Arg Lys Thr Ser Glu Arg
133   GGT GTG CGC GCG ACG AGA AAG ACT TCC GAG CGG
      CCA CAC GCG CGC TGC TCT TTC TGA AGG CTC GCC

      Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro
166   TCG CAA CCT CGA GGT AGA CGT CAG CCT ATC CCC
      AGC GTT GGA GCT CCA TCT GCA GTC GGA TAG GGG

      Lys Ala Arg Arg Pro Glu Gly Arg Thr Trp Ala
199   AAG GCT CGT CGG CCC GAG GGC AGG ACC TGG GCT
      TTC CGA GCA GCC GGG CTC CCG TCC TGG ACC CGA

      Gln Pro Gly Tyr Pro Trp Pro Leu Tyr Gly Asn
232   CAG CCC GGG TAC CCT TGG CCC CTC TAT GGC AAT
      GTC GGG CCC ATG GGA ACC GGG GAG ATA CCG TTA

      Glu Gly Cys Gly Trp Ala Gly Trp Leu Leu Ser
265   GAG GGC TGC GGG TGG GCG GGA TGG CTC CTG TCT
      CTC CCG ACG CCC ACC CGC CCT ACC GAG GAC AGA

      Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr
298   CCC CGT GGC TCT CGG CCT AGC TGG GGC CCC ACA
      GGG GCA CCG AGA GCC GGA TCG ACC CCG GGG TGT

      Asp Pro Arg Arg Arg Ser Arg Asn Leu Gly Lys
331   GAC CCC CGG CGT AGG TCG CGC AAT TTG GGT AAG
      CTG GGG GCC GCA TCC AGC GCG TTA AAC CCA TTC

      Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp
364   GTC ATC GAT ACC CTT ACG TGC GGC TTC GCC GAC
      CAG TAG CTA TGG GAA TGC ACG CCG AAG CGG CTG
```

FIG. 12-2

```
     Leu Met Gly Tyr Ile Pro Leu Val Gly Ala Pro
397  CTC ATG GGG TAC ATA CCG CTC GTC GGC GCC CCT
     GAG TAC CCC ATG TAT GGC GAG CAG CCG CGG GGA

     Leu Gly Gly Ala Ala Arg Ala Leu Ala His Gly
430  CTT GGA GGC GCT GCC AGG GCC CTG GCG CAT GGC
     GAA CCT CCG CGA CGG TCC CGG GAC CGC GTA CCG

     Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala
463  GTC CGG GTT CTG GAA GAC GGC GTG AAC TAT GCA
     CAG GCC CAA GAC CTT CTG CCG CAC TTG ATA CGT

     Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile
496  ACA GGG AAC CTT CCT GGT TGC TCT TTC TCT ATC
     TGT CCC TTG GAA GGA CCA ACG AGA AAG AGA TAG

     Phe Leu Leu Ala Leu Leu Ser Cys Leu Thr Val
529  TTC CTT CTG GCC CTG CTC TCT TGC TTG ACT GTG
     AAG GAA GAC CGG GAC GAG AGA ACG AAC TGA CAC

     Pro Ala Ser Ala Tyr Gln Val Arg Asn Ser Thr
562  CCC GCT TCG GCC TAC CAA GTG CGC AAC TCC ACG
     GGG CGA AGC CGG ATG GTT CAC GCG TTG AGG TGC

     Gly Leu Tyr His Val Thr Asn Asp Cys Pro Asn
595  GGG CTT TAC CAC GTC ACC AAT GAT TGC CCT AAC
     CCC GAA ATG GTG CAG TGG TTA CTA ACG GGA TTG

     Ser Ser Ile Val Tyr Glu Ala Ala Asp Ala Ile
628  TCG AGT ATT GTG TAC GAG GCG GCC GAT GCC ATC
     AGC TCA TAA CAC ATG CTC CGC CGG CTA CGG TAG

     Leu His Thr Pro Gly Cys Val Pro Cys Val Arg
661  CTG CAC ACT CCG GGG TGC GTC CCT TGC GTT CGT
     GAC GTG TGA GGC CCC ACG CAG GGA ACG CAA GCA
```

FIG. 12-3

```
        Glu Gly Asn Ala Ser Arg Cys Trp Val Ala Met
694     GAG GGC AAC GCC TCG AGG TGT TGG GTG GCG ATG
        CTC CCG TTG CGG AGC TCC ACA ACC CAC CGC TAC

        Thr Pro Thr Val Ala Thr Arg Asp Gly Lys Leu
727     ACC CCT ACG GTG GCC ACC AGG GAT GGC AAA CTC
        TGG GGA TGC CAC CGG TGG TCC CTA CCG TTT GAG

        Pro Ala Thr Gln Leu Arg Arg His Ile Asp Leu
760     CCC GCG ACG CAG CTT CGA CGT CAC ATC GAT CTG
        GGG CGC TGC GTC GAA GCT GCA GTG TAG CTA GAC

        Leu Val Gly Ser Ala Thr Leu Cys Ser Ala Leu
793     CTT GTC GGG AGC GCC ACC CTC TGT TCG GCC CTC
        GAA CAG CCC TCG CGG TGG GAG ACA AGC CGG GAG

        Tyr Val Gly Asp Leu Cys Gly Ser Val Phe Leu
826     TAC GTG GGG GAC CTA TGC GGG TCT GTC TTT CTT
        ATG CAC CCC CTG GAT ACG CCC AGA CAG AAA GAA

        Val Gly Gln Leu Phe Thr Phe Ser Pro Arg Arg
859     GTC GGC CAA CTG TTC ACC TTC TCT CCC AGG CGC
        CAG CCG GTT GAC AAG TGG AAG AGA GGG TCC GCG

        His Trp Thr Thr Gln Gly Cys Asn Cys Ser Ile
892     CAC TGG ACG ACG CAA GGT TGC AAT TGC TCT ATC
        GTG ACC TGC TGC GTT CCA ACG TTA ACG AGA TAG

        Tyr Pro Gly His Ile Thr Gly His Arg Met Ala
925     TAT CCC GGC CAT ATA ACG GGT CAC CGC ATG GCA
        ATA GGG CCG GTA TAT TGC CCA GTG GCG TAC CGT

        Trp Asp Met Met Met Asn Trp Ser Pro Thr Thr
958     TGG GAT ATG ATG ATG AAC TGG TCC CCT ACG ACG
        ACC CTA TAC TAC TAC TTG ACC AGG GGA TGC TGC
```

FIG. 12-4

```
          Ala Leu Val Met Ala Gln Leu Leu Arg Ile Pro
 991      GCG TTG GTA ATG GCT CAG CTG CTC CGG ATC CCA
          CGC AAC CAT TAC CGA GTC GAC GAG GCC TAG GGT

          Gln Ala Ile Leu Asp Met Ile Ala Gly Ala His
1024      CAA GCC ATC TTG GAC ATG ATC GCT GGT GCT CAC
          GTT CGG TAG AAC CTG TAC TAG CGA CCA CGA GTG

          Trp Gly Val Leu Ala Gly Ile Ala Tyr Phe Ser
1057      TGG GGA GTC CTG GCG GGC ATA GCG TAT TTC TCC
          ACC CCT CAG GAC CGC CCG TAT CGC ATA AAG AGG

          Met Val Gly Asn Trp Ala Lys Val Leu Val Val
1090      ATG GTG GGG AAC TGG GCG AAG GTC CTG GTA GTG
          TAC CAC CCC TTG ACC CGC TTC CAG GAC CAT CAC

          Leu Leu Leu Phe Ala Gly Val Asp Ala Glu Thr
1123      CTG CTG CTA TTT GCC GGC GTC GAC GCG GAA ACC
          GAC GAC GAT AAA CGG CCG CAG CTG CGC CTT TGG

          His Val Thr Gly Gly Ser Ala Gly His Thr Val
1156      CAC GTC ACC GGG GGA AGT GCC GGC CAC ACT GTG
          GTG CAG TGG CCC CCT TCA CGG CCG GTG TGA CAC

          Ser Gly Phe Val Ser Leu Leu Ala Pro Gly Ala
1189      TCT GGA TTT GTT AGC CTC CTC GCA CCA GGC GCC
          AGA CCT AAA CAA TCG GAG GAG CGT GGT CCG CGG

          Lys Gln Asn Val Gln Leu Ile Asn Thr Asn Gly
1222      AAG CAG AAC GTC CAG CTG ATC AAC ACC AAC GGC
          TTC GTC TTG CAG GTC GAC TAG TTG TGG TTG CCG

          Ser Trp His Leu Asn Ser Thr Ala Leu Asn Cys
1255      AGT TGG CAC CTC AAT AGC ACG GCC CTG AAC TGC
          TCA ACC GTG GAG TTA TCG TGC CGG GAC TTG ACG
```

FIG. 12-5

```
        Asn Asp Ser Leu Asn Thr Gly Trp Leu Ala Gly
1288    AAT GAT AGC CTC AAC ACC GGC TGG TTG GCA GGG
        TTA CTA TCG GAG TTG TGG CCG ACC AAC CGT CCC

        Leu Phe Tyr His His Lys Phe Asn Ser Ser Gly
1321    TTT TCT ATC ACC ACA AGT TCA ACT CTT CAG GCT
        GAA AAG ATA GTG GTG TTC AAG TTG AGA AGT CCG

        Cys Pro Glu Arg Leu Ala Ser Cys Arg Pro Leu
1354    GTC CTG AGA GGC TAG CCA GCT GCC GAC CCC CTT
        ACA GGA CTC TCC GAT CGG TCG ACG GCT GGG GAA

        Thr Asp Phe Asp Gln Gly Trp Gly Pro Ile Ser
1387    ACC GAT TTT GAC CAG GGC TGG GGC CCT ATC AGT
        TGG CTA AAA CTG GTC CCG ACC CCG GGA TAG TCA

        Tyr Ala Asn Gly Ser Gly Pro Asp Gln Arg Pro
1420    TAT GCC AAC GGA AGC GGC CCC GAC CAG CGC CCC
        ATA CGG TTG CCT TCG CCG GGG CTG GTC GCG GGG

        Tyr Cys Trp His Tyr Pro Pro Lys Pro Cys Gly
1453    TAC TGC TGG CAC TAC CCC CCA AAA CCT TGC GGT
        ATG ACG ACC GTG ATG GGG GGT TTT GGA ACG CCA

        Ile Val Pro Ala Lys Ser Val Cys Gly Pro Val
1486    ATT GTG CCC GCG AAG AGT GTG TGT GGT CCG GTA
        TAA CAC GGG CGC TTC TCA CAC ACA CCA GGC CAT

        Tyr Cys Phe Thr Pro Ser Pro Val Val Val Gly
1519    TAT TGC TTC ACT CCC AGC CCC GTG GTG GTG GGA
        ATA ACG AAG TGA GGG TCG GGG CAC CAC CAC CCT

        Thr Thr Asp Arg Ser Gly Ala Pro Thr Tyr Ser
1552    ACG ACC GAC AGG TCG GGC GCG CCC ACC TAC AGC
        TGC TGG CTG TCC AGC CCG CGC GGG TGG ATG TCG
```

FIG. 12-6

```
        Trp Gly Glu Asn Asp Thr Asp Val Phe Val Leu
1585    TGG GGT GAA AAT GAT ACG GAC GTC TTC GTC CTT
        ACC CCA CTT TTA CTA TGC CTG CAG AAG CAG GAA

        Asn Asn Thr Arg Pro Pro Leu Gly Asn Trp Phe
1618    AAC AAT ACC AGG CCA CCG CTG GGC AAT TGG TTC
        TTG TTA TGG TCC GGT GGC GAC CCG TTA ACC AAG

        Gly Cys Thr Trp Met Asn Ser Thr Gly Phe Thr
1651    GGT TGT ACC TGG ATG AAC TCA ACT GGA TTC ACC
        CCA ACA TGG ACC TAC TTG AGT TGA CCT AAG TGG

        Lys Val Cys Gly Ala Pro Pro Cys Val Ile Gly
1684    AAA GTG TGC GGA GCG CCT CCT TGT GTC ATC GGA
        TTT CAC ACG CCT CGC GGA GGA ACA CAG TAG CCT

        Gly Ala Gly Asn Asn Thr Leu His Cys Pro Thr
1717    GGG GCG GGC AAC AAC ACC CTG CAC TGC CCC ACT
        CCC CGC CCG TTG TTG TGG GAC GTG ACG GGG TGA

        Asp Cys Phe Arg Lys His Pro Asp Ala Thr Tyr
1750    GAT TGC TTC CGC AAG CAT CCG GAC GCC ACA_TAC
        CTA ACG AAG GCG TTC GTA GGC CTG CGG TGT ATG

        Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro
1783    TCT CGG TGC GGC TCC GGT CCC TGG ATC ACA CCC
        AGA GCC ACG CCG AGG CCA GGG ACC TAG TGT GGG

        Arg Cys Leu Val Asp Tyr Pro Tyr Arg Leu Trp
1816    AGG TGC CTG GTC GAC TAC CCG TAT AGG CTT TGG
        TCC ACG GAC CAG CTG ATG GGC ATA TCC GAA ACC

        His Tyr Pro Cys Thr Ile Asn Tyr Thr Ile Phe
1849_   CAT TAT CCT TGT ACC ATC AAC TAC ACC ATA TTT
        GTA ATA GGA ACA TGG TAG TTG ATG TGG TAT AAA
```

FIG. 12-7

```
        Lys Ile Arg Met Tyr Val Gly Gly Val Glu His
1882    AAA ATC AGG ATG TAC GTG GGA GGG GTC GAA CAC
        TTT TAG TCC TAC ATG CAC CCT CCC CAG CTT GTG

        Arg Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly
1915    AGG CTG GAA GCT GCC TGC AAC TGG ACG CGG GGC
        TCC GAC CTT CGA CGG ACG TTG ACC TGC GCC CCG

        Glu Arg Cys Asp Leu Glu Asp Arg Asp Arg Ser
1948    GAA CGT TGC GAT CTG GAA GAC AGG GAC AGG TCC
        CTT GCA ACG CTA GAC CTT CTG TCC CTG TCC AGG

        Glu Leu Ser Pro Leu Leu Leu Thr Thr Thr Gln
1981    GAG CTC AGC CCG TTA CTG CTG ACC ACT ACA CAG
        CTC GAG TCG GGC AAT GAC GAC TGG TGA TGT GTC

        Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu
2014    TGG CAG GTC CTC CCG TGT TCC TTC ACA ACC CTA
        ACC GTC CAG GAG GGC ACA AGG AAG TGT TGG GAT

        Pro Ala Leu Ser Thr Gly Leu Ile His Leu His
2047    CCA GCC TTG TCC ACC GGC CTC ATC CAC CTC CAC
        GGT CGG AAC AGG TGG CCG GAG TAG GTG GAG GTG

        Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr Gly
2080    CAG AAC ATT GTG GAC GTG CAG TAC TTG TAC GGG
        GTC TTG TAA CAC CTG CAC GTC ATG AAC ATG CCC

        Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys
2113    GTG GGG TCA AGC ATC GCG TCC TGG GCC ATT AAG
        CAC CCC AGT TCG TAG CGC AGG ACC CGG TAA TTC

        Trp Glu Tyr Val Val Leu Leu Phe Leu Leu Leu
2146    TGG GAG TAC GTC GTT CTC CTG TTC CTT CTG CTT
        ACC CTC ATG CAG CAA GAG GAC AAG GAA GAC GAA
```

FIG. 12-8

```
        Ala Asp Ala Arg Val Cys Ser Cys Leu Trp Met
2179    GCA GAC GCG CGC GTC TGC TCC TGC TTG TGG ATG
        CGT CTG CGC GCG CAG ACG AGG ACG AAC ACC TAC

        Met Leu Leu Ile Ser Gln Ala Glu Ala Ala Leu
2212    ATG CTA CTC ATA TCC CAA GCG GAG GCG GCT TTG
        TAC GAT GAG TAT AGG GTT CGC CTC CGC CGA AAC

        Glu Asn Leu Val Ile Leu Asn Ala Ala Ser Leu
2245    GAG AAC CTC GTA ATA CTT AAT GCA GCA TCC CTG
        CTC TTG GAG CAT TAT GAA TTA CGT CGT AGG GAC

        Ala Gly Thr His Gly Leu Val Ser Phe Leu Val
2278    GCC GGG ACG CAC GGT CTT GTA TCC TTC CTC GTG
        CGG CCC TGC GTG CCA GAA CAT AGG AAG GAG CAC

        Phe Phe Cys Phe Ala Trp Tyr Leu Lys Gly Lys
2311    TTC TTC TGC TTT GCA TGG TAT TTG AAG GGT AAG
        AAG AAG ACG AAA CGT ACC ATA AAC TTC CCA TTC

        Trp Val Pro Gly Ala Val Tyr Thr Phe Tyr Gly
2344    TGG GTG CCC GGA GCG GTC TAC ACC TTC TAC GGG
        ACC CAC GGG CCT CGC CAG ATG TGG AAG ATG CCC

        Met Trp Pro Leu Leu Leu Leu Leu Leu Ala Leu
2377    ATG TGG CCT CTC CTC CTG CTC CTG TTG GCG TTG
        TAC ACC GGA GAG GAG GAC GAG GAC AAC CGC AAC

        Pro Gln Arg Ala Tyr Ala Leu Asp Thr Glu Val
2410    CCC CAG CGG GCG TAC GCG CTG GAC ACG GAG GTG
        GGG GTC GCC CGC ATG CGC GAC CTG TGC CTC CAC

        Ala Ala Ser Cys Gly Gly Val Val Leu Val Gly
2443    GCC GCG TCG TGT GGC GGT GTT GTT CTC GTC GGG
        CGG CGC AGC ACA CCG CCA CAA CAA GAG CAG CCC
```

FIG. 12-9

```
        Leu Met Ala Leu Thr Leu Ser Pro Tyr Tyr Lys
2476    TTG ATG GCG CTG ACT CTG TCA CCA TAT TAC AAG
        AAC TAC CGC GAC TGA GAC AGT GGT ATA ATG TTC

        Arg Tyr Ile Ser Trp Cys Leu Trp Trp Leu Gln
2509    CGC TAT ATC AGC TGG TGC TTG TGG TGG CTT CAG
        GCG ATA TAG TCG ACC ACG AAC ACC ACC GAA GTC

        Tyr Phe Leu Thr Arg Val Glu Ala Gln Leu His
2542    TAT TTT CTG ACC AGA GTG GAA GCG CAA CTG CAC
        ATA AAA GAC TGG TCT CAC CTT CGC GTT GAC GTG

        Val Trp Ile Pro Pro Leu Asn Val Arg Gly Gly
2575    GTG TGG ATT CCC CCC CTC AAC GTC CGA GGG GGG
        CAC ACC TAA GGG GGG GAG TTG CAG GCT CCC CCC

        Arg Asp Ala Val Ile Leu Leu Met Cys Ala Val
2608    CGC GAC GCC GTC ATC TTA CTC ATG TGT GCT GTA
        GCG CTG CGG CAG TAG AAT GAG TAC ACA CGA CAT

        His Pro Thr Leu Val Phe Asp Ile Thr Lys Leu
2641    CAC CCG ACT CTG GTA TTT GAC ATC ACC AAA TTG
        GTG GGC TGA GAC CAT AAA CTG TAG TGG TTT AAC

        Leu Leu Ala Val Phe Gly Pro Leu Trp Ile Leu
2674    CTG CTG GCC GTC TTC GGA CCC CTT TGG ATT CTT
        GAC GAC CGG CAG AAG CCT GGG GAA ACC TAA GAA

        Gln Ala Ser Leu Leu Lys Val Pro Tyr Phe Val
2707    CAA GCC AGT TTG CTT AAA GTA CCC TAC TTT GTG
        GTT CGG TCA AAC GAA TTT CAT GGG ATG AAA CAC

        Arg Val Gln Gly Leu Leu Arg Phe Cys Ala Leu
2740    CGC GTC CAA GGC CTT CTC CGG TTC TGC GCG TTA
        GCG CAG GTT CCG GAA GAG GCC AAG ACG CGC AAT
```

FIG. 12-10

```
        Ala Arg Lys Met Ile Gly Gly His Tyr Val Gln
2773    GCG CGG AAG ATG ATC GGA GGC CAT TAC GTG CAA
        CGC GCC TTC TAC TAG CCT CCG GTA ATG CAC GTT

        Met Val Ile Ile Lys Leu Gly Ala Leu Thr Gly
2806    ATG GTC ATC ATT AAG TTA GGG GCG CTT ACT GGC
        TAC CAG TAG TAA TTC AAT CCC CGC GAA TGA CCG

        Thr Tyr Val Tyr Asn His Leu Thr Pro Leu Arg
2839    ACC TAT GTT TAT AAC CAT CTC ACT CCT CTT CGG
        TGG ATA CAA ATA TTG GTA GAG TGA GGA GAA GCC

        Asp Trp Ala His Asn Gly Leu Arg Asp Leu Ala
2872    GAC TGG GCG CAC AAC GGC TTG CGA GAT CTG GCC
        CTG ACC CGC GTG TTG CCG AAC GCT CTA GAC CGG

        Val Ala Val Glu Pro Val Val Phe Ser Gln Met
2905    GTG GCT GTA GAG CCA GTC GTC TTC TCC CAA ATG
        CAC CGA CAT CTC GGT CAG CAG AAG AGG GTT TAC

        Glu Thr Lys Leu Ile Thr Trp Gly Ala Asp Thr
2938    GAG ACC AAG CTC ATC ACG TGG GGG GCA GAT ACC
        CTC TGG TTC GAG TAG TGC ACC CCC CGT CTA TGG

        Ala Ala Cys Gly Asp Ile Ile Asn Gly Leu Pro
2971    GCC GCG TGC GGT GAC ATC ATC AAC GGC TTG CCT
        CGG CGC ACG CCA CTG TAG TAG TTG CCG AAC GGA

        Val Ser Ala Arg Arg Gly Arg Glu Ile Leu Leu
3004    GTT TCC GCC CGC AGG GGC CGG GAG ATA CTG CTC
        CAA AGG CGG GCG TCC CCG GCC CTC TAT GAC GAG

        Gly Pro Ala Asp Gly Met Val Ser Lys Gly Trp
3037    GGG CCA GCC GAT GGA ATG GTC TCC AAG GGG TGG
        CCC GGT CGG CTA CCT TAC CAG AGG TTC CCC ACC
```

FIG. 12-11

```
        Arg Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln
3070    AGG TTG CTG GCG CCC ATC ACG GCG TAC GCC CAG
        TCC AAC GAC CGC GGG TAG TGC CGC ATG CGG GTC

        Gln Thr Arg Gly Leu Leu Gly Cys Ile Ile Thr
3103    CAG ACA AGG GGC CTC CTA GGG TGC ATA ATC ACC
        GTC TGT TCC CCG GAG GAT CCC ACG TAT TAG TGG

        Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu
3136    AGC CTA ACT GGC CGG GAC AAA AAC CAA GTG GAG
        TCG GAT TGA CCG GCC CTG TTT TTG GTT CAC CTC

        Gly Glu Val Gln Ile Val Ser Thr Ala Ala Gln
3169    GGT GAG GTC CAG ATT GTG TCA ACT GCT GCC CAA
        CCA CTC CAG GTC TAA CAC AGT TGA CGA CGG GTT

        Thr Phe Leu Ala Thr Cys Ile Asn Gly Val Cys
3202    ACC TTC CTG GCA ACG TGC ATC AAT GGG GTG TGC
        TGG AAG GAC CGT TGC ACG TAG TTA CCC CAC ACG

        Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
3235    TGG ACT GTC TAC CAC GGG GCC GGA ACG AGG ACC
        ACC TGA CAG ATG GTG CCC CGG CCT TGC TCC TGG

        Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met
3268    ATC GCG TCA CCC AAG GGT CCT GTC ATC CAG ATG
        TAG CGC AGT GGG TTC CCA GGA CAG TAG GTC TAC

        Tyr Thr Asn Val Asp Gln Asp Leu Val Gly Trp
3301    TAT ACC AAT GTA GAC CAA GAC CTT GTG GGC TGG
        ATA TGG TTA CAT CTG GTT CTG GAA CAC CCG ACC

        Pro Ala Pro Gln Gly Ser Arg Ser Leu Thr Pro
3334    CCC GCT CCG CAA GGT AGC CGC TCA TTG ACA CCC
        GGG CGA GGC GTT CCA TCG GCG AGT AAC TGT GGG
```

FIG. 12-12

```
        Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val
3367    TGC ACT TGC GGC TCC TCG GAC CTT TAC CTG GTC
        ACG TGA ACG CCG AGG AGC CTG GAA ATG GAC CAG

        Thr Arg His Ala Asp Val Ile Pro Val Arg Arg
3400    ACG AGG CAC GCC GAT GTC ATT CCC GTG CGC CGG
        TGC TCC GTG CGG CTA CAG TAA GGG CAC GCG GCC

        Arg Gly Asp Ser Arg Gly Ser Leu Leu Ser Pro
3433    CGG GGT GAT AGC AGG GGC AGC CTG CTG TCG CCC
        GCC CCA CTA TCG TCC CCG TCG GAC GAC AGC GGG

        Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly
3466    CGG CCC ATT TCC TAC TTG AAA GGC TCC TCG GGG
        GCC GGG TAA AGG ATG AAC TTT CCG AGG AGC CCC

        Gly Pro Leu Leu Cys Pro Ala Gly His Ala Val
3499    GGT CCG CTG TTG TGC CCC GCG GGG CAC GCC GTG
        CCA GGC GAC AAC ACG GGG CGC CCC GTG CGG CAC

        Gly Ile Phe Arg Ala Ala Val Cys Thr Arg Gly
3532    GGC ATA TTT AGG GCC GCG GTG TGC ACC CGT GGA
        CCG TAT AAA TCC CGG CGC CAC ACG TGG GCA CCT

        Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu
3565    GTG GCT AAG GCG GTG GAC TTT ATC CCT GTG GAG
        CAC CGA TTC CGC CAC CTG AAA TAG GGA CAC CTC

        Asn Leu Glu Thr Thr Met Arg Ser Pro Val Phe
3598    AAC CTA GAG ACA ACC ATG AGG TCC CCG GTG TTC
        TTG GAT CTC TGT TGG TAC TCC AGG GGC CAC AAG

        Thr Asp Asn Ser Ser Pro Pro Val Val Pro Gln
3631    ACG GAT AAC TCC TCT CCA CCA GTA GTG CCC CAG
        TGC CTA TTG AGG AGA GGT GGT CAT CAC GGG GTC
```

FIG. 12-13

```
        Ser Phe Gln Val Ala His Leu His Ala Pro Thr
3664    AGC TTC CAG GTG GCT CAC CTC CAT GCT CCC ACA
        TCG AAG GTC CAC CGA GTG GAG GTA CGA GGG TGT

        Gly Ser Gly Lys Ser Thr Lys Val Pro Ala Ala
3697    GGC AGC GGC AAA AGC ACC AAG GTC CCG GCT GCA
        CCG TCG CCG TTT TCG TGG TTC CAG GGC CGA CGT

        Tyr Ala Ala Gln Gly Tyr Lys Val Leu Val Leu
3730    TAT GCA GCT CAG GGC TAT AAG GTG CTA GTA CTC
        ATA CGT CGA GTC CCG ATA TTC CAC GAT CAT GAG

        Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly
3763    AAC CCC TCT GTT GCT GCA ACA CTG GGC TTT GGT
        TTG GGG AGA CAA CGA CGT TGT GAC CCG AAA CCA

        Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro
3796    GCT TAC ATG TCC AAG GCT CAT GGG ATC GAT CCT
        CGA ATG TAC AGG TTC CGA GTA CCC TAG CTA GGA

        Asn Ile Arg Thr Gly Val Arg Thr Ile Thr Thr
3829    AAC ATC AGG ACC GGG GTG AGA ACA ATT ACC ACT
        TTG TAG TCC TGG CCC CAC TCT TGT TAA TGG TGA

        Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly Lys
3862    GGC AGC CCC ATC ACG TAC TCC ACC TAC GGC AAG
        CCG TCG GGG TAG TGC ATG AGG TGG ATG CCG TTC

        Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala
3895    TTC CTT GCC GAC GGC GGG TGC TCG GGG GGC GCT
        AAG GAA CGG CTG CCG CCC ACG AGC CCC CCG CGA

        Tyr Asp Ile Ile Ile Cys Asp Glu Cys His Ser
3928    TAT GAC ATA ATA ATT TGT GAC GAG TGC CAC TCC
        ATA CTG TAT TAT TAA ACA CTG CTC ACG GTG AGG
```

FIG. 12-14

```
        Thr Asp Ala Thr Ser Ile Leu Gly Ile Gly Thr
3961    ACG GAT GCC ACA TCC ATC TTG GGC ATC GGC ACT
        TGC CTA CGG TGT AGG TAG AAC CCG TAG CCG TGA

        Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg
3994    GTC CTT GAC CAA GCA GAG ACT GCG GGG GCG AGA
        CAG GAA CTG GTT CGT CTC TGA CGC CCC CGC TCT

        Leu Val Val Leu Ala Thr Ala Thr Pro Pro Gly
4027    CTG GTT GTG CTC GCC ACC GCC ACC CCT CCG GGC
        GAC CAA CAC GAG CGG TGG CGG TGG GGA GGC CCG

        Ser Val Thr Val Pro His Pro Asn Ile Glu Glu
4060    TCC GTC ACT GTG CCC CAT CCC AAC ATC GAG GAG
        AGG CAG TGA CAC GGG GTA GGG TTG TAG CTC CTC

        Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe
4093    GTT GCT CTG TCC ACC ACC GGA GAG ATC CCT TTT
        CAA CGA GAC AGG TGG TGG CCT CTC TAG GGA AAA

        Tyr Gly Lys Ala Ile Pro Leu Glu Val Ile Lys
4126    TAC GGC AAG GCT ATC CCC CTC GAA GTA ATC AAG
        ATG CCG TTC CGA TAG GGG GAG CTT CAT TAG TTC

        Gly Gly Arg His Leu Ile Phe Cys His Ser Lys
4159    GGG GGG AGA CAT CTC ATC TTC TGT CAT TCA AAG
        CCC CCC TCT GTA GAG TAG AAG ACA GTA AGT TTC

        Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val
4192    AAG AAG TGC GAC GAA CTC GCC GCA AAG CTG GTC
        TTC TTC ACG CTG CTT GAG CGG CGT TTC GAC CAG

        Ala Leu Gly Ile Asn Ala Val Ala Tyr Tyr Arg
4225    GCA TTG GGC ATC AAT GCC GTG GCC TAC TAC CGC
        CGT AAC CCG TAG TTA CGG CAC CGG ATG ATG GCG
```

FIG. 12-15

```
        Gly Leu Asp Val Ser Val Ile Pro Thr Ser Gly
4258    GGT CTT GAC GTG TCC GTC ATC CCG ACC AGC GGC
        CCA GAA CTG CAC AGG CAG TAG GGC TGG TCG CCG

        Asp Val Val Val Val Ala Thr Asp Ala Leu Met
4291    GAT GTT GTC GTC GTG GCA ACC GAT GCC CTC ATG
        CTA CAA CAG CAG CAC CGT TGG CTA CGG GAG TAC

        Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile
4324    ACC GGC TAT ACC GGC GAC TTC GAC TCG GTG ATA
        TGG CCG ATA TGG CCG CTG AAG CTG AGC CAC TAT

        Asp Cys Asn Thr Cys Val Thr Gln Thr Val Asp
4357    GAC TGC AAT ACG TGT GTC ACC CAG ACA GTC GAT
        CTG ACG TTA TGC ACA CAG TGG GTC TGT CAG CTA

        Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr
4390    TTC AGC CTT GAC CCT ACC TTC ACC ATT GAG ACA
        AAG TCG GAA CTG GGA TGG AAG TGG TAA CTC TGT

        Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr
4423    ATC ACG CTC CCC CAG GAT GCT GTC TCC CGC ACT
        TAG TGC GAG GGG GTC CTA CGA CAG AGG GCG TGA

        Gln Arg Arg Gly Arg Thr Gly Arg Gly Lys Pro
4456    CAA CGT CGG GGC AGG ACT GGC AGG GGG AAG CCA
        GTT GCA GCC CCG TCC TGA CCG TCC CCC TTC GGT

        Gly Ile Tyr Arg Phe Val Ala Pro Gly Glu Arg
4489    GGC ATC TAC AGA TTT GTG GCA CCG GGG GAG CGC
        CCG TAG ATG TCT AAA CAC CGT GGC CCC CTC GCG

        Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys
4522    CCC TCC GGC ATG TTC GAC TCG TCC GTC CTC TGT
        GGG AGG CCG TAC AAG CTG AGC AGG CAG GAG ACA
```

FIG. 12-16

```
        Glu Cys Tyr Asp Ala Gly Cys Ala Trp Tyr Glu
4555    GAG TGC TAT GAC GCA GGC TGT GCT TGG TAT GAG
        CTC ACG ATA CTG CGT CCG ACA CGA ACC ATA CTC

        Leu Thr Pro Ala Glu Thr Thr Val Arg Leu Arg
4588    CTC ACG CCC GCC GAG ACT ACA GTT AGG CTA CGA
        GAG TGC GGG CGG CTC TGA TGT CAA TCC GAT GCT

        Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys
4621    GCG TAC ATG AAC ACC CCG GGG CTT CCC GTG TGC
        CGC ATG TAC TTG TGG GGC CCC GAA GGG CAC ACG

        Gln Asp His Leu Glu Phe Trp Glu Gly Val Phe
4654    CAG GAC CAT CTT GAA TTT TGG GAG GGC GTC TTT
        GTC CTG GTA GAA CTT AAA ACC CTC CCG CAG AAA

        Thr Gly Leu Thr His Ile Asp Ala His Phe Leu
4687    ACA GGC CTC ACT CAT ATA GAT GCC CAC TTT CTA
        TGT CCG GAG TGA GTA TAT CTA CGG GTG AAA GAT

        Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro
4720    TCC CAG ACA AAG CAG AGT GGG GAG AAC CTT CCT
        AGG GTC TGT TTC GTC TCA CCC CTC TTG GAA GGA

        Tyr Leu Val Ala Tyr Gln Ala Thr Val Cys Ala
4753    TAC CTG GTA GCG TAC CAA GCC ACC GTG TGC GCT
        ATG GAC CAT CGC ATG GTT CGG TGG CAC ACG CGA

        Arg Ala Gln Ala Pro Pro Pro Ser Trp Asp Gln
4786    AGG GCT CAA GCC CCT CCC CCA TCG TGG GAC CAG
        TCC CGA GTT CGG GGA GGG GGT AGC ACC CTG GTC

        Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
4819    ATG TGG AAG TGT TTG ATT CGC CTC AAG CCC ACC
        TAC ACC TTC ACA AAC TAA GCG GAG TTC GGG TGG
```

FIG. 12-17

```
        Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu
4852    CTC CAT GGG CCA ACA CCC CTG CTA TAC AGA CTG
        GAG GTA CCC GGT TGT GGG GAC GAT ATG TCT GAC

        Gly Ala Val Gln Asn Glu Ile Thr Leu Thr His
4885    GGC GCT GTT CAG AAT GAA ATC ACC CTG ACG CAC
        CCG CGA CAA GTC TTA CTT TAG TGG GAC TGC GTG

        Pro Val Thr Lys Tyr Ile Met Thr Cys Met Ser
4918    CCA GTC ACC AAA TAC ATC ATG ACA TGC ATG TCG
        GGT CAG TGG TTT ATG TAG TAC TGT ACG TAC AGC

        Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val
4951    GCC GAC CTG GAG GTC GTC ACG AGC ACC TGG GTG
        CGG CTG GAC CTC CAG CAG TGC TCG TGG ACC CAC

        Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala
4984    CTC GTT GGC GGC GTC CTG GCT GCT TTG GCC GCG
        GAG CAA CCG CCG CAG GAC CGA CGA AAC CGG CGC

        Tyr Cys Leu Ser Thr Gly Cys Val Val Ile Val
5017    TAT TGC CTG TCA ACA GGC TGC GTG GTC ATA GTG
        ATA ACG GAC AGT TGT CCG ACG CAC CAG TAT CAC

        Gly Arg Val Val Leu Ser Gly Lys Pro Ala Ile
5050    GGC AGG GTC GTC TTG TCC GGG AAG CCG GCA ATC
        CCG TCC CAG CAG AAC AGG CCC TTC GGC CGT TAG

        Ile Pro Asp Arg Glu Val Leu Tyr Arg Glu Phe
5083    ATA CCT GAC AGG GAA GTC CTC TAC CGA GAG TTC
        TAT GGA CTG TCC CTT CAG GAG ATG GCT CTC AAG

        Asp Glu Met Glu Glu Cys Ser Gln His Leu Pro
5116    GAT GAG ATG GAA GAG TGC TCT CAG CAC TTA CCG
        CTA CTC TAC CTT CTC ACG AGA GTC GTG AAT GGC
```

FIG. 12-18

```
        Tyr Ile Glu Gln Gly Met Met Leu Ala Glu Gln
5149    TAC ATC GAG CAA GGG ATG ATG CTC GCC GAG CAG
        ATG TAG CTC GTT CCC TAC TAC GAG CGG CTC GTC

        Phe Lys Gln Lys Ala Leu Gly Leu Leu Gln Thr
5182    TTC AAG CAG AAG GCC CTC GGC CTC CTG CAG ACC
        AAG TTC GTC TTC CGG GAG CCG GAG GAC GTC TGG

        Ala Ser Arg Gln Ala Glu Val Ile Ala Pro Ala
5215    GCG TCC CGT CAG GCA GAG GTT ATC GCC CCT GCT
        CGC AGG GCA GTC CGT CTC CAA TAG CGG GGA CGA

        Val Gln Thr Asn Trp Gln Lys Leu Glu Thr Phe
5248    GTC CAG ACC AAC TGG CAA AAA CTC GAG ACC TTC
        CAG GTC TGG TTG ACC GTT TTT GAG CTC TGG AAG

        Trp Ala Lys His Met Trp Asn Phe Ile Ser Gly
5281    TGG GCG AAG CAT ATG TGG AAC TTC ATC AGT GGG
        ACC CGC TTC GTA TAC ACC TTG AAG TAG TCA CCC

        Ile Gln Tyr Leu Ala Gly Leu Ser Thr Leu Pro
5314    ATA CAA TAC TTG GCG GGC TTG TCA ACG CTG CCT
        TAT GTT ATG AAC CGC CCG AAC AGT TGC GAC GGA

        Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe
5347    GGT AAC CCC GCC ATT GCT TCA TTG ATG GCT TTT
        CCA TTG GGG CGG TAA CGA AGT AAC TAC CGA AAA

        Thr Ala Ala Val Thr Ser Pro Leu Thr Thr Ser
5380    ACA GCT GCT GTC ACC AGC CCA CTA ACC ACT AGC
        TGT CGA CGA CAG TGG TCG GGT GAT TGG TGA TCG

        Gln Thr Leu Leu Phe Asn Ile Leu Gly Gly Trp
5413    CAA ACC CTC CTC TTC AAC ATA TTG GGG GGG TGG
        GTT TGG GAG GAG AAG TTG TAT AAC CCC CCC ACC
```

FIG. 12-19

```
        Val Ala Ala Gln Leu Ala Ala Pro Gly Ala Ala
5446    GTG GCT GCC CAG CTC GCC GCC CCC GGT GCC GCT
        CAC CGA CGG GTC GAG CGG CGG GGG CCA CGG CGA

        Thr Ala Phe Val Gly Ala Gly Leu Ala Gly Ala
5479    ACT GCC TTT GTG GGC GCT GGC TTA GCT GGC GCC
        TGA CGG AAA CAC CCG CGA CCG AAT CGA CCG CGG

        Ala Ile Gly Ser Val Gly Leu Gly Lys Val Leu
5512    GCC ATC GGC AGT GTT GGA CTG GGG AAG GTC CTC
        CGG TAG CCG TCA CAA CCT GAC CCC TTC CAG GAG

        Ile Asp Ile Leu Ala Gly Tyr Gly Ala Gly Val
5545    ATA GAC ATC CTT GCA GGG TAT GGC GCG GGC GTG
        TAT CTG TAG GAA CGT CCC ATA CCG CGC CCG CAC

        Ala Gly Ala Leu Val Ala Phe Lys Ile Met Ser
5578    GCG GGA GCT CTT GTG GCA TTC AAG ATC ATG AGC
        CGC CCT CGA GAA CAC CGT AAG TTC TAG TAC TCG

        Gly Glu Val Pro Ser Thr Glu Asp Leu Val Asn
5611    GGT GAG GTC CCC TCC ACG GAG GAC CTG GTC AAT
        CCA CTC CAG GGG AGG TGC CTC CTG GAC CAG TTA

        Leu Leu Pro Ala Ile Leu Ser Pro Gly Ala Leu
5644    CTA CTG CCC GCC ATC CTC TCG CCC GGA GCC CTC
        GAT GAC GGG CGG TAG GAG AGC GGG CCT CGG GAG

        Val Val Gly Val Val Cys Ala Ala Ile Leu Arg
5677    GTA GTC GGC GTG GTC TGT GCA GCA ATA CTG CGC
        CAT CAG CCG CAC CAG ACA CGT CGT TAT GAC GCG

        Arg His Val Gly Pro Gly Glu Gly Ala Val Gln
5710    CGG CAC GTT GGC CCG GGC GAG GGG GCA GTG CAG
        GCC GTG CAA CCG GGC CCG CTC CCC CGT CAC GTC
```

FIG. 12-20

```
        Trp Met Asn Arg Leu Ile Ala Phe Ala Ser Arg
5743    TGG ATG AAC CGG CTG ATA GCC TTC GCC TCC CGG
        ACC TAC TTG GCC GAC TAT CGG AAG CGG AGG GCC

        Gly Asn His Val Ser Pro Thr His Tyr Val Pro
5776    GGG AAC CAT GTT TCC CCC ACG CAC TAC GTG CCG
        CCC TTG GTA CAA AGG GGG TGC GTG ATG CAC GGC

        Glu Ser Asp Ala Ala Ala Arg Val Thr Ala Ile
5809    GAG AGC GAT GCA GCT GCC CGC GTC ACT GCC ATA
        CTC TCG CTA CGT CGA CGG GCG CAG TGA CGG TAT

        Leu Ser Ser Leu Thr Val Thr Gln Leu Leu Arg
5842    CTC AGC AGC CTC ACT GTA ACC CAG CTC CTG AGG
        GAG TCG TCG GAG TGA CAT TGG GTC GAG GAC TCC

        Arg Leu His Gln Trp Ile Ser Ser Glu Cys Thr
5875    CGA CTG CAC CAG TGG ATA AGC TCG GAG TGT ACC
        GCT GAC GTG GTC ACC TAT TCG AGC CTC ACA TGG

        Thr Pro Cys Ser Gly Ser Trp Leu Arg Asp Ile
5908    ACT CCA TGC TCC GGT TCC TGG CTA AGG GAC ATC
        TGA GGT ACG AGG CCA AGG ACC GAT TCC CTG TAG

        Trp Asp Trp Ile Cys Glu Val Leu Ser Asp Phe
5941    TGG GAC TGG ATA TGC GAG GTG TTG AGC GAC TTT
        ACC CTG ACC TAT ACG CTC CAC AAC TCG CTG AAA

        Lys Thr Trp Leu Lys Ala Lys Leu Met Pro Gln
5974    AAG ACC TGG CTA AAA GCT AAG CTC ATG CCA CAG
        TTC TGG ACC GAT TTT CGA TTC GAG TAC GGT GTC

        Leu Pro Gly Ile Pro Phe Val Ser Cys Gln Arg
6007    CTG CCT GGG ATC CCC TTT GTG TCC TGC CAG CGC
        GAC GGA CCC TAG GGG AAA CAC AGG ACG GTC GCG
```

FIG. 12-21

```
        Gly Tyr Lys Gly Val Trp Arg Val Asp Gly Ile
6040    GGG TAT AAG GGG GTC TGG CGA GTG GAC GGC ATC
        CCC ATA TTC CCC CAG ACC GCT CAC CTG CCG TAG

        Met His Thr Arg Cys His Cys Gly Ala Glu Ile
6073    ATG CAC ACT CGC TGC CAC TGT GGA GCT GAG ATC
        TAC GTG TGA GCG ACG GTG ACA CCT CGA CTC TAG

        Thr Gly His Val Lys Asn Gly Thr Met Arg Ile
6106    ACT GGA CAT GTC AAA AAC GGG ACG ATG AGG ATC
        TGA CCT GTA CAG TTT TTG CCC TGC TAC TCC TAG

        Val Gly Pro Arg Thr Cys Arg Asn Met Trp Ser
6139    GTC GGT CCT AGG ACC TGC AGG AAC ATG TGG AGT
        CAG CCA GGA TCC TGG ACG TCC TTG TAC ACC TCA

        Gly Thr Phe Pro Ile Asn Ala Tyr Thr Thr Gly
6172    GGG ACC TTC CCC ATT AAT GCC TAC ACC ACG GGC
        CCC TGG AAG GGG TAA TTA CGG ATG TGG TGC CCG

        Pro Cys Thr Pro Leu Pro Ala Pro Asn Tyr Thr
6205    CCC TGT ACC CCC CTT CCT GCG CCG AAC TAC ACG
        GGG ACA TGG GGG GAA GGA CGC GGC TTG ATG TGC

        Phe Ala Leu Trp Arg Val Ser Ala Glu Glu Tyr
6238    TTC GCG CTA TGG AGG GTG TCT GCA GAG GAA TAT
        AAG CGC GAT ACC TCC CAC AGA CGT CTC CTT ATA

        Val Glu Ile Arg Gln Val Gly Asp Phe His Tyr
6271    GTG GAG ATA AGG CAG GTG GGG GAC TTC CAC TAC
        CAC CTC TAT TCC GTC CAC CCC CTG AAG GTG ATG

        Val Thr Gly Met Thr Thr Asp Asn Leu Lys Cys
6304    GTG ACG GGT ATG ACT ACT GAC AAT CTC AAA TGC
        CAC TGC CCA TAC TGA TGA CTG TTA GAG TTT ACG
```

FIG. 12-22

```
        Pro Cys Gln Val Pro Ser Pro Glu Phe Phe Thr
6337    CCG TGC CAG GTC CCA TCG CCC GAA TTT TTC ACA
        GGC ACG GTC CAG GGT AGC GGG CTT AAA AAG TGT

        Glu Leu Asp Gly Val Arg Leu His Arg Phe Ala
6370    GAA TTG GAC GGG GTG CGC CTA CAT AGG TTT GCG
        CTT AAC CTG CCC CAC GCG GAT GTA TCC AAA CGC

        Pro Pro Cys Lys Pro Leu Leu Arg Glu Glu Val
6403    CCC CCC TGC AAG CCC TTG CTG CGG GAG GAG GTA
        GGG GGG ACG TTC GGG AAC GAC GCC CTC CTC CAT

        Ser Phe Arg Val Gly Leu His Glu Tyr Pro Val
6436    TCA TTC AGA GTA GGA CTC CAC GAA TAC CCG GTA
        AGT AAG TCT CAT CCT GAG GTG CTT ATG GGC CAT

        Gly Ser Gln Leu Pro Cys Glu Pro Glu Pro Asp
6469    GGG TCG CAA TTA CCT TGC GAG CCC GAA CCG GAC
        CCC AGC GTT AAT GGA ACG CTC GGG CTT GGC CTG

        Val Ala Val Leu Thr Ser Met Leu Thr Asp Pro
6502    GTG GCC GTG TTG ACG TCC ATG CTC ACT GAT CCC
        CAC CGG CAC AAC TGC AGG TAC GAG TGA CTA GGG

        Ser His Ile Thr Ala Glu Ala Ala Gly Arg Arg
6535    TCC CAT ATA ACA GCA GAG GCG GCC GGG CGA AGG
        AGG GTA TAT TGT CGT CTC CGC CGG CCC GCT TCC

        Leu Ala Arg Gly Ser Pro Pro Ser Val Ala Ser
6568    TTG GCG AGG GGA TCA CCC CCC TCT GTG GCC AGC
        AAC CGC TCC CCT AGT GGG GGG AGA CAC CGG TCG

        Ser Ser Ala Ser Gln Leu Ser Ala Pro Ser Leu
6601    TCC TCG GCT AGC CAG CTA TCC GCT CCA TCT CTC
        AGG AGC CGA TCG GTC GAT AGG CGA GGT AGA GAG
```

FIG. 12-23

```
        Lys Ala Thr Cys Thr Ala Asn His Asp Ser Pro
6634    AAG GCA ACT TGC ACC GCT AAC CAT GAC TCC CCT
        TTC CGT TGA ACG TGG CGA TTG GTA CTG AGG GGA

        Asp Ala Glu Leu Ile Glu Ala Asn Leu Leu Trp
6667    GAT GCT GAG CTC ATA GAG GCC AAC CTC CTA TGG
        CTA CGA CTC GAG TAT CTC CGG TTG GAG GAT ACC

        Arg Gln Glu Met Gly Gly Asn Ile Thr Arg Val
6700    AGG CAG GAG ATG GGC GGC AAC ATC ACC AGG GTT
        TCC GTC CTC TAC CCG CCG TTG TAG TGG TCC CAA

        Glu Ser Glu Asn Lys Val Val Ile Leu Asp Ser
6733    GAG TCA GAA AAC AAA GTG GTG ATT CTG GAC TCC
        CTC AGT CTT TTG TTT CAC CAC TAA GAC CTG AGG

        Phe Asp Pro Leu Val Ala Glu Glu Asp Glu Arg
6766    TTC GAT CCG CTT GTG GCG GAG GAG GAC GAG CGG
        AAG CTA GGC GAA CAC CGC CTC CTC CTG CTC GCC

        Glu Ile Ser Val Pro Ala Glu Ile Leu Arg Lys
6799    GAG ATC TCC GTA CCC GCA GAA ATC CTG CGG AAG
        CTC TAG AGG CAT GGG CGT CTT TAG GAC GCC TTC

        Ser Arg Arg Phe Ala Gln Ala Leu Pro Val Trp
6832    TCT CGG AGA TTC GCC CAG GCC CTG CCC GTT TGG
        AGA GCC TCT AAG CGG GTC CGG GAC GGG CAA ACC

        Ala Arg Pro Asp Tyr Asn Pro Pro Leu Val Glu
6865    GCG CGG CCG GAC TAT AAC CCC CCG CTA GTG GAG
        CGC GCC GGC CTG ATA TTG GGG GGC GAT CAC CTC

        Thr Trp Lys Lys Pro Asp Tyr Glu Pro Pro Val
6898    ACG TGG AAA AAG CCC GAC TAC GAA CCA CCT GTG
        TGC ACC TTT TTC GGG CTG ATG CTT GGT GGA CAC
```

FIG. 12-24

```
        Val His Gly Cys Pro Leu Pro Pro Pro Lys Ser
6931    GTC CAT GGC TGT CCG CTT CCA CCT CCA AAG TCC
        CAG GTA CCG ACA GGC GAA GGT GGA GGT TTC AGG

        Pro Pro Val Pro Pro Pro Arg Lys Lys Arg Thr
6964    CCT CCT GTG CCT CCG CCT CGG AAG AAG CGG ACG
        GGA GGA CAC GGA GGC GGA GCC TTC TTC GCC TGC

        Val Val Leu Thr Glu Ser Thr Leu Ser Thr Ala
6997    GTG GTC CTC ACT GAA TCA ACC CTA TCT ACT GCC
        CAC CAG GAG TGA CTT AGT TGG GAT AGA TGA CGG

        Leu Ala Glu Leu Ala Thr Arg Ser Phe Gly Ser
7030    TTG GCC GAG CTC GCC ACC AGA AGC TTT GGC AGC
        AAC CGG CTC GAG CGG TGG TCT TCG AAA CCG TCG

        Ser Ser Thr Ser Gly Ile Thr Gly Asp Asn Thr
7063    TCC TCA ACT TCC GGC ATT ACG GGC GAC AAT ACG
        AGG AGT TGA AGG CCG TAA TGC CCG CTG TTA TGC

        Thr Thr Ser Ser Glu Pro Ala Pro Ser Gly Cys
7096    ACA ACA TCC TCT GAG CCC GCC CCT TCT GGC TGC
        TGT TGT AGG AGA CTC GGG CGG GGA AGA CCG ACG

        Pro Pro Asp Ser Asp Ala Glu Ser Tyr Ser Ser
7129    CCC CCC GAC TCC GAC GCT GAG TCC TAT TCC TCC
        GGG GGG CTG AGG CTG CGA CTC AGG ATA AGG AGG

        Met Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro
7162    ATG CCC CCC CTG GAG GGG GAG CCT GGG GAT CCG
        TAC GGG GGG GAC CTC CCC CTC GGA CCC CTA GGC

        Asp Leu Ser Asp Gly Ser Trp Ser Thr Val Ser
7195    GAT CTT AGC GAC GGG TCA TGG TCA ACG GTC AGT
        CTA GAA TCG CTG CCC AGT ACC AGT TGC CAG TCA
```

FIG. 12-25

```
          Ser Glu Ala Asn Ala Glu Asp Val Val Cys Cys
7228      AGT GAG GCC AAC GCG GAG GAT GTC GTG TGC TGC
          TCA CTC CGG TTG CGC CTC CTA CAG CAC ACG ACG

          Ser Met Ser Tyr Ser Trp Thr Gly Ala Leu Val
7261      TCA ATG TCT TAC TCT TGG ACA GGC GCA CTC GTC
          AGT TAC AGA ATG AGA ACC TGT CCG CGT GAG CAG

          Thr Pro Cys Ala Ala Glu Glu Gln Lys Leu Pro
7294      ACC CCG TGC GCC GCG GAA GAA CAG AAA CTG CCC
          TGG GGC ACG CGG CGC CTT CTT GTC TTT GAC GGG

          Ile Asn Ala Leu Ser Asn Ser Leu Leu Arg His
7327      ATC AAT GCA CTA AGC AAC TCG TTG CTA CGT CAC
          TAG TTA CGT GAT TCG TTG AGC AAC GAT GCA GTG

          His Asn Leu Val Tyr Ser Thr Thr Ser Arg Ser
7360      CAC AAT TTG GTG TAT TCC ACC ACC TCA CGC AGT
          GTG TTA AAC CAC ATA AGG TGG TGG AGT GCG TCA

          Ala Cys Gln Arg Gln Lys Lys Val Thr Phe Asp
7393      GCT TGC CAA AGG CAG AAG AAA GTC ACA TTT GAC
          CGA ACG GTT TCC GTC TTC TTT CAG TGT AAA CTG

          Arg Leu Gln Val Leu Asp Ser His Tyr Gln Asp
7426      AGA CTG CAA GTT CTG GAC AGC CAT TAC CAG GAC
          TCT GAC GTT CAA GAC CTG TCG GTA ATG GTC CTG

          Val Leu Lys Glu Val Lys Ala Ala Ala Ser Lys
7459      GTA CTC AAG GAG GTT AAA GCA GCG GCG TCA AAA
          CAT GAG TTC CTC CAA TTT CGT CGC CGC AGT TTT

          Val Lys Ala Asn Leu Leu Ser Val Glu Glu Ala
7492      GTG AAG GCT AAC TTG CTA TCC GTA GAG GAA GCT
          CAC TTC CGA TTG AAC GAT AGG CAT CTC CTT CGA
```

FIG. 12-26

```
          Cys Ser Leu Thr Pro Pro His Ser Ala Lys Ser
7525      TGC AGC CTG ACG CCC CCA CAC TCA GCC AAA TCC
          ACG TCG GAC TGC GGG GGT GTG AGT CGG TTT AGG

          Lys Phe Gly Tyr Gly Ala Lys Asp Val Arg Cys
7558      AAG TTT GGT TAT GGG GCA AAA GAC GTC CGT TGC
          TTC AAA CCA ATA CCC CGT TTT CTG CAG GCA ACG

          His Ala Arg Lys Ala Val Thr His Ile Asn Ser
7591      CAT GCC AGA AAG GCC GTA ACC CAC ATC AAC TCC
          GTA CGG TCT TTC CGG CAT TGG GTG TAG TTG AGG

          Val Trp Lys Asp Leu Leu Glu Asp Asn Val Thr
7624      GTG TGG AAA GAC CTT CTG GAA GAC AAT GTA ACA
          CAC ACC TTT CTG GAA GAC CTT CTG TTA CAT TGT

          Pro Ile Asp Thr Thr Ile Met Ala Lys Asn Glu
7657      CCA ATA GAC ACT ACC ATC ATG GCT AAG AAC GAG
          GGT TAT CTG TGA TGG TAG TAC CGA TTC TTG CTC

          Val Phe Cys Val Gln Pro Glu Lys Gly Gly Arg
7690      GTT TTC TGC GTT CAG CCT GAG AAG GGG GGT CGT
          CAA AAG ACG CAA GTC GGA CTC TTC CCC CCA GCA

          Lys Pro Ala Arg Leu Ile Val Phe Pro Asp Leu
7723      AAG CCA GCT CGT CTC ATC GTG TTC CCC GAT CTG
          TTC GGT CGA GCA GAG TAG CAC AAG GGG CTA GAC

          Gly Val Arg Val Cys Glu Lys Met Ala Leu Tyr
7756      GGC GTG CGC GTG TGC GAA AAG ATG GCT TTG TAC
          CCG CAC GCG CAC ACG CTT TTC TAC CGA AAC ATG

          Asp Val Val Thr Lys Leu Pro Leu Ala Val Met
7789      GAC GTG GTT ACA AAG CTC CCC TTG GCC GTG ATG
          CTG CAC CAA TGT TTC GAG GGG AAC CGG CAC TAC
```

FIG. 12-27

```
          Gly Ser Ser Tyr Gly Phe Gln Tyr Ser Pro Gly
7822      GGA AGC TCC TAC GGA TTC CAA TAC TCA CCA GGA
          CCT TCG AGG ATG CCT AAG GTT ATG AGT GGT CCT

          Gln Arg Val Glu Phe Leu Val Gln Ala Trp Lys
7855      CAG CGG GTT GAA TTC CTC GTG CAA GCG TGG AAG
          GTC GCC CAA CTT AAG GAG CAC GTT CGC ACC TTC

          Ser Lys Lys Thr Pro Met Gly Phe Ser Tyr Asp
7888      TCC AAG AAA ACC CCA ATG GGG TTC TCG TAT GAT
          AGG TTC TTT TGG GGT TAC CCC AAG AGC ATA CTA

          Thr Arg Cys Phe Asp Ser Thr Val Thr Glu Ser
7921      ACC CGC TGC TTT GAC TCC ACA GTC ACT GAG AGC
          TGG GCG ACG AAA CTG AGG TGT CAG TGA CTC TCG

          Asp Ile Arg Thr Glu Glu Ala Ile Tyr Gln Cys
7954      GAC ATC CGT ACG GAG GAG GCA ATC TAC CAA TGT
          CTG TAG GCA TGC CTC CTC CGT TAG ATG GTT ACA

          Cys Asp Leu Asp Pro Gln Ala Arg Val Ala Ile
7987      TGT GAC CTC GAC CCC CAA GCC CGC GTG GCC ATC
          ACA CTG GAG CTG GGG GTT CGG GCG CAC CGG TAG

          Lys Ser Leu Thr Glu Arg Leu Tyr Val Gly Gly
8020      AAG TCC CTC ACC GAG AGG CTT TAT GTT GGG GGC
          TTC AGG GAG TGG CTC TCC GAA ATA CAA CCC CCG

          Pro Leu Thr Asn Ser Arg Gly Glu Asn Cys Gly
8053      CCT CTT ACC AAT TCA AGG GGG GAG AAC TGC GGC
          GGA GAA TGG TTA AGT TCC CCC CTC TTG ACG CCG

          Tyr Arg Arg Cys Arg Ala Ser Gly Val Leu Thr
8086      TAT CGC AGG TGC CGC GCG AGC GGC GTA CTG ACA
          ATA GCG TCC ACG GCG CGC TCG CCG CAT GAC TGT
```

FIG. 12-28

```
        Thr Ser Cys Gly Asn Thr Leu Thr Cys Tyr Ile
8119    ACT AGC TGT GGT AAC ACC CTC ACT TGC TAC ATC
        TGA TCG ACA CCA TTG TGG GAG TGA ACG ATG TAG

        Lys Ala Arg Ala Ala Cys Arg Ala Ala Gly Leu
8152    AAG GCC CGG GCA GCC TGT CGA GCC GCA GGG CTC
        TTC CGG GCC CGT CGG ACA GCT CGG CGT CCC GAG

        Gln Asp Cys Thr Met Leu Val Cys Gly Asp Asp
8185    CAG GAC TGC ACC ATG CTC GTG TGT GGC GAC GAC
        GTC CTG ACG TGG TAC GAG CAC ACA CCG CTG CTG

        Leu Val Val Ile Cys Glu Ser Ala Gly Val Gln
8218    TTA GTC GTT ATC TGT GAA AGC GCG GGG GTC CAG
        AAT CAG CAA TAG ACA CTT TCG CGC CCC CAG GTC

        Glu Asp Ala Ala Ser Leu Arg Ala Phe Thr Glu
8251    GAG GAC GCG GCG AGC CTG AGA GCC TTC ACG GAG
        CTC CTG CGC CGC TCG GAC TCT CGG AAG TGC CTC

        Ala Met Thr Arg Tyr Ser Ala Pro Pro Gly Asp
8284    GCT ATG ACC AGG TAC TCC GCC CCC CCT GGG GAC
        CGA TAC TGG TCC ATG AGG CGG GGG GGA CCC CTG

        Pro Pro Gln Pro Glu Tyr Asp Leu Glu Leu Ile
8317    CCC CCA CAA CCA GAA TAC GAC TTG GAG CTC ATA
        GGG GGT GTT GGT CTT ATG CTG AAC CTC GAG TAT

        Thr Ser Cys Ser Ser Asn Val Ser Val Ala His
8350    ACA TCA TGC TCC TCC AAC GTG TCA GTC GCC CAC
        TGT AGT ACG AGG AGG TTG CAC AGT CAG CGG GTG

        Asp Gly Ala Gly Lys Arg Val Tyr Tyr Leu Thr
8383    GAC GGC GCT GGA AAG AGG GTC TAC TAC CTC ACC
        CTG CCG CGA CCT TTC TCC CAG ATG ATG GAG TGG
```

FIG. 12-29

```
        Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala Ala
8416    CGT GAC CCT ACA ACC CCC CTC GCG AGA GCT GCG
        GCA CTG GGA TGT TGG GGG GAG CGC TCT CGA CGC

        Trp Glu Thr Ala Arg His Thr Pro Val Asn Ser
8449    TGG GAG ACA GCA AGA CAC ACT CCA GTC AAT TCC
        ACC CTC TGT CGT TCT GTG TGA GGT CAG TTA AGG

        Trp Leu Gly Asn Ile Ile Met Phe Ala Pro Thr
8482    TGG CTA GGC AAC ATA ATC ATG TTT GCC CCC ACA
        ACC GAT CCG TTG TAT TAG TAC AAA CGG GGG TGT

        Leu Trp Ala Arg Met Ile Leu Met Thr His Phe
8515    CTG TGG GCG AGG ATG ATA CTG ATG ACC CAT TTC
        GAC ACC CGC TCC TAC TAT GAC TAC TGG GTA AAG

        Phe Ser Val Leu Ile Ala Arg Asp Gln Leu Glu
8548    TTT AGC GTC CTT ATA GCC AGG GAC CAG CTT GAA
        AAA TCG CAG GAA TAT CGG TCC CTG GTC GAA CTT

        Gln Ala Leu Asp Cys Glu Ile Tyr Gly Ala Cys
8581    CAG GCC CTC GAT TGC GAG ATC TAC GGG GCC TGC
        GTC CGG GAG CTA ACG CTC TAG ATG CCC CGG ACG

        Tyr Ser Ile Glu Pro Leu Asp Leu Pro Pro Ile
8614    TAC TCC ATA GAA CCA CTT GAT CTA CCT CCA ATC
        ATG AGG TAT CTT GGT GAA CTA GAT GGA GGT TAG

        Ile Gln Arg Leu His Gly Leu Ser Ala Phe Ser
8647    ATT CAA AGA CTC CAT GGC CTC AGC GCA TTT TCA
        TAA GTT TCT GAG GTA CCG GAG TCG CGT AAA AGT

        Leu His Ser Tyr Ser Pro Gly Glu Ile Asn Arg
8680    CTC CAC AGT TAC TCT CCA GGT GAA ATT AAT AGG
        GAG GTG TCA ATG AGA GGT CCA CTT TAA TTA TCC
```

FIG. 12-30

```
        Val Ala Ala Cys Leu Arg Lys Leu Gly Val Pro
8713    GTG GCC GCA TGC CTC AGA AAA CTT GGG GTA CCG
        CAC CGG CGT ACG GAG TCT TTT GAA CCC CAT GGC

        Pro Leu Arg Ala Trp Arg His Arg Ala Arg Ser
8746    CCC TTG CGA GCT TGG AGA CAC CGG GCC CGG AGC
        GGG AAC GCT CGA ACC TCT GTG GCC CGG GCC TCG

        Val Arg Ala Arg Leu Leu Ala Arg Gly Gly Arg
8779    GTC CGC GCT AGG CTT CTG GCC AGA GGA GGC AGG
        CAG GCG CGA TCC GAA GAC CGG TCT CCT CCG TCC

        Ala Ala Ile Cys Gly Lys Tyr Leu Phe Asn Trp
8812    GCT GCC ATA TGT GGC AAG TAC CTC TTC AAC TGG
        CGA CGG TAT ACA CCG TTC ATG GAG AAG TTG ACC

        Ala Val Arg Thr Lys Leu Lys
8845    GCA GTA AGA ACA AAG CTC AAA C
        CGT CAT TCT TGT TTC GAG TTT G
```

FIG. 12-31

primer J159S

J1 ACTGCCCTGAACTGCAATGA

PT G

			Ser	Leu	Lys	Thr	Gly	Phe	Leu	Ala	Ala
J1	1	C	TCC	CTC	AAA	ACT	GGG	TTT	CTT	GCC	GCG
PT		T	AG		C	C	C	GG	T G	A	G
					Asn			Trp			Gly
			Leu	Phe	Tyr	Thr	His	Lys	Phe	Asn	Ala
J1	29		CTG	TTC	TAC	ACA	CAC	AAG	TTC	AAC	GCG
PT		T		T	CAC					T	T
					His						Ser
			Ser	Gly	Cys	Pro	Glu	Arg	Met	Ala	Ser
J1	56		TCC	GGA	TGC	CCG	GAG	CGC	ATG	GCC	AGC
PT			A	C	T	T		A G	C A		
									Leu		
			Cys	Arg	Ser	Ile	Asp	Lys	Phe	Asp	Gln
J1	83		TGT	CGC	TCC	ATT	GAC	AAG	TTC	GAC	CAG
PT			C	A	C	C	AC	G T	T		
					Pro	Leu	Thr	Asp			
			Gly	Trp	Gly	Pro	Ile	Thr	Tyr	Ala	Gln
J1	110		GGA	TGG	GGT	CCC	ATC	ACC	TAT	GCT	CAA
PT			C		C	T		GT		C	A C
								Ser			Asn
			Pro	Asp	Asn	Ser	Asp	Gln	Arg	Pro	Tyr
J1	137		CCT	GAC	AAC	TCG	GAC	CAG	AGG	CCG	TAT
PT			GGA	AG	GG	C C			C C	C	C
			Gly	Ser	Gly	Pro					
			Cys	Trp	His	Tyr	Ala	Pro	Arg	Gln	Cys
J1	164		TGC	TGG	CAC	TAC	GCA	CCT	CGA	CAG	TGT
PT							C C	A	AA	CT	C
							Pro		Lys	Pro	

FIG. 13-1

```
                    Gly Ile Val Pro Ala Ser Gln Val Cys
J1        191       GGT ATC GTA CCC GCG TCG CAG GTG TGC
PT                      T   G               AA  AGT   T
                                            Lys Ser

                    Gly Pro Val Tyr Cys Phe Thr Pro Ser
J1        218       GGT CCA GTG TAT TGC TTC ACC CCA AGC
PT                      G   A                   T   C

                    Pro Val Val Val Gly Thr Thr Asp Arg
J1        245       CCT GTT GTA GTG GGG ACG ACC GAT CGT
PT                    C   G   G       A           C A G

                    Phe Gly Ala Pro Thr Tyr Asn Trp Gly
J1        272       TTC GGC GCC CCT ACG TAT AAC TGG GGG
PT                    CG      G   C   C   C  G       T
                    Ser                       Ser

                    Asp Asn Glu Thr Asp Val Leu Leu Leu
J1        299       GAC AAT GAG ACG GAC GTG CTG CTC CTA
PT                      A       T           C T C G   T
                    Glu     Asp                 Phe Val

                    Asn Asn Thr Arg Pro Pro His Gly Asn
J1        326       AAC AAC ACG CGG CCC CCG CAC GGC AAC
PT                        T   C A     A       TG      T
                                              Leu

                    Trp Phe Gly Cys Thr
J1        353       TGG TTC GGC TGT ACA
PT                            T       CTGGATGAACTCAACTGGATT
                                            primer 199A
```

Nucleotide Match: 259/367 (70.6%)
Amino Acid Match (stringent): 93/122 (76.2%)
(relaxed): 111/122 (91.0%)

FIG. 13-2

Prototype HCV (PT) sequences different from Japanese HCV (J1) are shown.
Relaxed amino acid match: Gly=Ala=Pro=Ser=Thr, Asp=Glu, Asn=Gln,
Aug=Lys=His, Leu=Ile=Val=Met, Phe=Trp=Tyr.
Underline, different amino acid in relaxed matching.

FIG. 13-3

Core to NS1 vs. HCV-1

J1								Pro	Leu	Val
HCV-1							T	CCG	CTC	GTC
							A	---	---	---
		Gly	Ala	Pro	Leu	Gly	Gly	Ala	Ala	Arg
J1	11	GGC	GCC	CCC	TTA	GGG	GGC	GCT	GCC	AGG
		---	---	--T	C-T	--A	---	---	---	---
		Ala	Leu	Ala	His	Gly	Val	Arg	Val	Leu
J1	38	GCC	CTG	GCA	CAT	GGT	GTC	CGG	GTT	CTG
		---	---	--G	---	--C	---	---	---	---
		Glu	Asp	Gly	Val	Asn	Tyr	Ala	Thr	Gly
J1	65	GAG	GAC	GGC	GTG	AAC	TAT	GCA	ACA	GGG
		--A	---	---	---	---	---	---	---	---
		Asn	Leu	Pro	Gly	Cys	Ser	Phe	Ser	Ile
J1	92	AAT	TTG	CCC	GGT	TGC	TCT	TTC	TCT	ATC
		--C	C-T	--T	---	---	---	---	---	---
		Phe	Leu	Leu	Ala	Leu	Leu	Ser	Cys	Leu
J1	119	TTC	CTC	TTG	GCT	CTG	CTG	TCC	TGT	TTG
		---	--T	C--	---	---	--C	--T	--C	---
		Thr	Ile	Pro	Ala	Ser	Ala	Tyr	Glu	Val
J1	146	ACC	ATC	CCA	GCT	TCC	GCT	TAT	GAA	GTG
		--T	G-G	--C	---	--G	--C	--C	C--	---
		Val							Gln	
		Arg	Asn	Val	Ser	Gly	Ile	Tyr	His	Val
J1	173	CGC	AAC	GTG	TCC	GGG	ATA	TAC	CAT	GTC
		---	---	TCC	A-G	---	C-T	---	--C	---
				Ser	Thr		Leu			
		Thr	Asn	Asp	Cys	Ser	Asn	Ser	Ser	Ile
J1	200	ACA	AAC	GAC	TGC	TCC	AAC	TCA	AGC	ATT
		--C	--T	--T	---	C-T	---	--G	--T	---
						Pro				

FIG. 14-1

```
                Val Tyr Glu Ala Ala Asp Val Ile Met
J1        227   GTG TAT GAG GCG GCG GAC GTG ATC ATG
                --- --C --- --- --C --T -CC --- C--
                                      Ala      Leu

                His Ala Pro Gly Cys Val Pro Cys Val
J1        254   CAT GCC CCC GGG TGC GTG CCC TGC GTT
                --C A-T --G --- --- --C --T --- ---
                    Thr

                Arg Glu Asn Asn Ser Ser Arg Cys Trp
J1        281   CGG GAG AAC AAT TCC TCC CGT TGC TGG
                --T --- GG- --C G-- --G A-G --T ---
                        Gly      Ala

                Val Ala Leu Thr Pro Thr Leu Ala Ala
J1        308   GTA GCG CTC ACT CCC ACG CTC GCG GCC
                --A --- A-G --C --T --- G-G --C A--
                        Met                  Val  Thr

                Arg Asn Ala Ser Val Pro Thr Thr Thr
J1        335   AGG AAT GCC AGC GTC CCC ACT ACG ACA
                --- G-- -G- -AA C-- --- G-G --- CAG
                    Asp Gly Lys Leu          Ala
Gln

                Leu Arg Arg His Val Asp Leu Leu Val
J1        362   TTA CGA CGC CAC GTC GAC TTG CTC GTT
                C-T --- --T --- A-- --T C-- --T --C
                                Ile

                Gly Thr Ala Ala Phe Cys Ser Ala Met
J1        389   GGG ACG GCT GCT TTC TGC TCC GCT ATG
                --- -GC --C A-C C-- --T --G --C C-C
                    Ser      Thr Leu              Leu

                Tyr Val Gly Asp Leu Cys Gly Ser Val
J1        416   TAC GTG GGG GAT CTC TGC GGA TCT GTT
                --- --- --- --C --A --- --G --- --C
```

FIG. 14-2

```
                Phe Leu Ile Ser Gln Leu Phe Thr Phe
J1      443     TTC CTC ATC TCC CAG CTG TTC ACC TTC
                --T --T G-- GG- --A --- --- --- ---
                    Val Gly

                Ser Pro Arg Arg His Glu Thr Val Gln
J1      470     TCG CCT CGC CGG CAT GAG ACA GTA CAG
                --T --C A-G --C --C TG- --G ACG --A
                                    Trp     Thr

                Asp Cys Asn Cys Ser Ile Tyr Pro Gly
J1      497     GAC TGC AAC TGC TCA ATC TAT CCC GGC
                -GT --- --T --- --T --- --- --- ---
                Gly

                His Val Ser Gly His Arg Met Ala Trp
J1      524     CAC GTA TCA GGC CAT CGC ATG GCT TGG
                --T A-- A-G --T --C --- --- --A ---
                    Ile Thr

                Asp Met Met Met Asn Trp Ser Pro Thr
J1      551     GAT ATG ATG ATG AAC TGG TCG CCC ACG
                --- --- --- --- --- --- --C --T ---

                Ala Ala Leu Val Val Ser Gln Leu Leu
J1      578     GCA GCC TTA GTG GTG TCG CAG TTA CTC
                A-G --G --G --A A-- G-T --- C-G ---
                Thr             Met Ala

                Arg Ile Pro Gln Ala Val Met Asp Met
J1      605     CGG ATC CCA CAA GCT GTC ATG GAC ATG
                --- --- --- --- --C A-- T-- --- ---
                                    Ile Leu

                Val Ala Gly Ala His Trp Gly Val Leu
J1      632     GTG GCG GGG GCC CAC TGG GGA GTC CTA
                A-C --T --T --T --- --- --- --- --G
                Ile
```

FIG. 14-3

```
                Ala Gly Leu Ala Tyr Tyr Ser Met Val
J1        659   GCG GGC CTT GCC TAC TAT TCC ATG GTG
                --- --- A-A --G --T -TC --- --- ---
                    Ile         Phe

                Gly Asn Trp Ala Lys Val Leu Ile Val
J1        686   GGG AAC TGG GCT AAG GTT TTG ATT GTG
                --- --- --- --G --- --C C-- G-A ---
                                        Val

                Met Leu Leu Phe Ala Gly Val Asp Gly
J1        713   ATG CTA CTC TTT GCC GGC GTT GAC GGG
                C-- --G --A --- --- --- --C --- -C-
                Leu                         Ala

                His Thr Arg Val Thr Gly Gly Val Gln
J1        740   CAT ACC CGC GTG ACG GGG GGG GTG CAA
                G-A --- -A- --C --C --- --A AGT GCC
                Glu     His                 Ser Ala

                Gly His Val Thr Ser Thr Leu Thr Ser
J1        767   GGC CAC GTC ACC TCT ACA CTC ACG TCC
                --- --- ACT GTG --- GGA T-T GTT AG-
                        Thr Val     Gly Phe Val

                Leu Phe Arg Pro Gly Ala Ser Gln Lys
J1        794   CTC TTT AGA CCT GGG GCG TCC CAG AAA
                --- C-C GC- --A --C --C AAG C-- --C
                    Leu Ala             Lys     Asn

                Ile Gln Leu Val Asn Thr Asn Gly Ser
J1        821   ATT CAG CTT GTA AAC ACC AAT GGC AGT
                G-C --- --G A-C --- --- --C --- ---
                Val         Ile

                Trp His Ile Asn Arg Thr Ala Leu Asn
J1        848   TGG CAT ATC AAC AGG ACT GCC CTG AAC
                --- --C C-- --T --C --G --- --- ---
                                Ser
```

FIG. 14-4

```
                  Cys Asn Asp Ser Leu Gln Thr Gly Phe
J1        875     TGC AAT GAC TCC CTC CAA ACT GGG TTC
                  --- --- --T AG- --- A-C --C --C -GG
                                      Asn         Trp

                  Leu Ala Ala
J1        902     CTT GCC GCG CTG TTC TAC ACA CAC AAG
                  T-G --A -G- --T --- --T CAC C-- ---
                        Gly                 His

J1        929     TTC AAC GCG TCC GGA TGC CCG GAG CGC
                  --- --- T-T --A --C --T --T --- A-G
                        Ser

                                      Ser Ile Asp Lys
                  ATG GCC AGC TGT CGC TCC ATT GAC AAG
J1        956     C-A --- --- --C --A C-- C-- AC- G-T
                  Leu                 Pro Leu Thr Asp

                  Phe Asp Gln Gly Trp Gly Pro Ile Thr
J1         983    TTC GAC CAG GGA TGG GGT CCC ATC ACC
                  --T --- --- --C --- --C --T --- -GT
                                                  Ser

                  Tyr Ala Gln Pro Asp Asn Ser Asp Gln
J1        1010    TAT GCT CAA CCT GAC AAC TCG GAC CAG
                  --- --C AAC GGA AGC GG- C-C --- ---
                        Asn Gly Ser Gly Pro

                  Arg Pro Tyr Cys Trp His Tyr Ala Pro
J1        1037    AGG CCG TAT TGC TGG CAC TAC GCA CCT
                  C-C --C --C --- --- --- --- C-C --A
                                              Pro

                  Arg Gln Cys Gly Ile Val Pro Ala Ser
J1        1064    CGA CAG TGT GGT ATC GTA CCC GCG TCG
                  AA- -CT --C --- --T --G --- --- AA-
                  Lys Pro                         Lys
```

FIG. 14-5

```
                Gln Val Cys Gly Pro Val Tyr Cys Phe
J1      1091    CAG GTG TGC GGT CCA GTG TAT TGC TTC
                AGT --- --T --- --G --A --- --- ---
                Ser

                Thr Pro Ser Pro Val Val Val Gly Thr
J1      1118    ACC CCA AGC CCT GTT GTA GTG GGG ACG
                --T --C --- --C --G --G --- --A ---

                Thr Asp Arg Phe Gly Ala Pro Thr Tyr
J1      1145    ACC GAT CGT TTC GGC GCC CCT ACG TAT
                --- --- A-G -CG --- --G --C --C --C
                        Ser

                Asn Trp Gly Asp Asn Glu Thr Asp Val
J1      1172    AAC TGG GGG GAC AAT GAG ACG GAC GTG
                -G- --- --T --A --- --T --- --- --C
                Ser     Glu     Asp

                Leu Leu Leu Asn Asn Thr Arg Pro Pro
J1      1199    CTG CTC CTA AAC AAC ACG CGG CCC CCG
                T-C G-- --T --- --T --C A-- --A ---
                Phe Val

                His Gly Asn Trp Phe Gly Cys Thr
J1      1226    CAC GGC AAC TGG TTC GGC TGT ACA
                -TG --- --T --- --- --T --- --
                Leu
```

FIG. 14-6

```
                    Gly Asn Trp Phe Gly Cys Thr Trp Met
          1    TG GGC AAC TGG TTC GGC TGT ACA TGG ATG
J1         
HCV-1       -- --- --T --- --- --T --- --C --- ---

                    Asn Ser Thr Gly Phe Thr Lys Thr Cys
         30    AAT AGC ACT GGG TTC ACC AAG ACG TGC
J1         
HCV-1       --C TCA --- --A --- --- --A GT- ---
                                        Val

                    Gly Gly Pro Pro Cys Asn Ile Gly Gly
         57    GGA GGC CCC CCG TGT AAC ATC GGG GGG
J1         
HCV-1       --- -CG --T --T --- GT- --- --A ---
                                Val

                    Val Gly Asn Asn Thr Leu Thr Cys Pro
         84    GTC GGC AAC AAC ACC TTG ACC TGC CCC
J1         
HCV-1       -CG --- --- --- --- C-- CA- --- ---
            Ala                     His

                    Thr Asp Cys Phe Arg Lys Thr Pro Thr
        111    ACG GAC TGC TTC CGG AAG ACC CCG ACG
J1         
HCV-1       --T --T --- --- --C --- CAT --- GAC
                                    His     Asp

                    Ala Thr Tyr Thr Lys Cys Gly Ser Gly
        138    GCC ACT TAC ACA AAA TGT GGT TCG GGC
J1         
HCV-1       --- --A --- T-T CGG --C --C --C --T
                    Ser Arg

                    Pro Trp Leu Thr Pro Arg Cys Leu Val
        165    CCT TGG TTG ACA CCT AGG TGC TTG GTT
J1         
HCV-1       --C --- A-C --- --C --- --- C-- --C
                    Ile

                    Asp Tyr Pro Tyr Arg Leu Trp His Tyr
        192    GAC TAC CCA TAC AGG CTC TGG CAC TAC
J1         
HCV-1       --- --- --G --T --- --T --- --T --T
```

FIG. 15-1

		Pro	Cys	Thr	Val	Asn	Phe	Thr	Ile	Phe
J1	219	CCC	TGC	ACT	GTC	AAC	TTT	ACC	ATC	TTC
HCV-1		--T	--T	--C	A--	---	-AC	---	--A	--T
					Ile		Tyr			
		Lys	Val	Arg	Met	Tyr	Val	Gly	Gly	Val
J1	246	AAG	GTT	AGG	ATG	TAT	GTG	GGG	GGC	GTG
HCV-1		--A	A-C	---	---	--C	---	--A	--G	--C
			Ile							
		Glu	His							
J1	273	GAG	CAC							
HCV-1		--A	---							

FIG. 15-2

SUBSTITUTE SHEET

C200 region sequence vs. HCV-1

```
                                                          Asn Met Ser
                                                          AAT ATG TCC
C200                                          3799        AAT ATG TCC
HCV-1   3781 ACA CTG GGC TTT GGT GCT T-C --- ---
             Thr Leu Gly Phe Gly Ala Tyr

             Lys Ala His Gly Thr Asp Pro Asn Ile
C200    3808 AAG GCA CAT GGC ACC GAC CCC AAC ATC
HCV-1        --- --T --- --G -T- --T --T --- ---
                                 Ile

             Arg Thr Gly Val Arg Thr Ile Thr Thr
C200    3835 AGA ACT GGG GTA AGG ACC ATC ACC ACA
HCV-1        --G --C --- --G --A --A --T --- --T

             Gly Ala Pro Ile Thr Tyr Ser Thr Tyr
C200    3862 GGT GCC CCC ATT ACG TAC TCC ACC TAT
HCV-1        --C AG- --- --C --- --- --- --- --C
                 Ser

             Arg Lys Phe Leu Ala Asp Gly Gly Cys
C200    3889 CGC AAG TTC CTT GCC GAC GGT GGT TGC
HCV-1        G-- --- --- --- --- --- --C --G ---
             Gly

             Ser Gly Gly Ala Tyr Asp Ile Ile
C200    3916 TCC GGG GGC GCC TAT GAC ATC ATA A
HCV-1        --G --- --- --T --- --- --A --- -TT
                                             Ile

HCV-1   3943 TGT GAC GAG TGC CAC TCC ACG GAT GCC
             Cys Asp Glu Cys His Ser Thr Asp Ala

HCV-1   3970 ACA TCC ATC TTG GGC ATC GGC ACT GTC
             Thr Ser Ile Leu Gly Ile Gly Thr Val
```

FIG. 16-1

```
HCV-1   3997 CTT GAC CAA GCA GAG ACT GCG GGG GCG
             Leu Asp Gln Ala Glu Thr Ala Gly Ala

HCV-1   4024 AGA CTG GTT GTG CTC GCC ACC GCC ACC
             Arg Leu Val Val Leu Ala Thr Ala Thr

HCV-1   4051 CCT CCG GGC TCC GTC ACT GTG CCC CAT
             Pro Pro Gly Ser Val Thr Val Pro His

HCV-1   4078 CCC AAC ATC GAG GAG GTT GCT CTG TCC
             Pro Asn Ile Glu Glu Val Ala Leu Ser

HCV-1   4105 ACC ACC GGA GAG ATC CCT TTT TAC GGC
             Thr Thr Gly Glu Ile Pro Phe Tyr Gly

                 Ser Ile Pro Ile Glu Ala Ile Lys
C200    4132   A AGC ATC CCC ATC GAG GCC ATC AAG
HCV-1          AAG GCT --- --- C-- --A -TA --- ---
               Lys Ala                   Val

                 Gly Gly Arg His Leu Ile Phe Cys His
C200    4159 GGG GGA AGG CAT CTC ATC TTC TGC CAT
HCV-1        --- --G --A --- --- --- --- --T ---

                 Ser Lys Lys Lys Cys Asp Glu Leu Ala
C200    4186 TCC AAG AAG AAG TGT GAC GAG CTC GCC
HCV-1        --A --- --- --- --C --- --A --- ---

                 Ala Lys Leu Ser Ala Leu Gly Leu Asn
C200    4213 GCA AAG CTG TCA GCC CTC GGA CTC AAT
HCV-1        --- --- --- GTC --A T-G --C A-- ---
                         Val                Ile

                 Ala Val Ala Tyr Tyr Arg Gly Leu Asp
C200    4240 GCC GTG GCG TAT TAC CGC GGT CTT GAT
HCV-1        --- --- --C --C --- --- --- --- --C
```

FIG. 16-2

```
                Val Ser Val Ile Pro Thr Ser Gly Asp
C200    4267    GTG TCC GTC ATA CCA ACT AGC GGA GAC
HCV-1           --- --- --- --C --G --C --- --C --T

                Val Val Val Val Ala Thr Asp
C200    4294    GTC GTT GTC GTG GCA ACA GAC GC 4316
HCV-1           --T --C --- --- --- --C --T --C CTC

HCV-1   4321    ATG ACC GGC TAT ACC GGC GAC TTC GAC
                Met Thr Gly Tyr Thr Gly Asp Phe Asp

HCV-1   4348    TCG GTG ATA GAC TGC AAT ACG TGT GTC
                Ser Val Ile Asp Cys Asn Thr Cys Val

HCV-1   4375    ACC CAG ACA GTC GAT TTC AGC CTT GAC
                Thr Gln Thr Val Asp Phe Ser Leu Asp

HCV-1   4402    CCT ACC TTC ACC ATT GAG ACA ATC ACG
                Pro Thr Phe Thr Ile Glu Thr Ile Thr

HCV-1   4429    CTC CCC CAG GAT GCT GTC TCC CGC ACT
                Leu Pro Gln Asp Ala Val Ser Arg Thr

HCV-1   4456    CAA CGT CGG GGC AGG ACT GGC AGG GGG
                Gln Arg Arg Gly Arg Thr Gly Arg Gly

HCV-1   4483    AAG CCA GGC ATC TAC AGA TTT GTG GCA
                Lys Pro Gly Ile Tyr Arg Phe Val Ala

HCV-1   4510    CCG GGG GAG CGC CCC TCC GGC ATG TTC
                Pro Gly Glu Arg Pro Ser Gly Met Phe

HCV-1   4537    GAC TCG TCC GTC CTC TGT GAG TGC TAT
                Asp Ser Ser Val Leu Cys Glu Cys Tyr
```

FIG. 16-3

```
HCV-1   4564 GAC GCA GGC TGT GCT TGG TAT GAG CTC
             Asp Ala Gly Cys Ala Trp Tyr Glu Leu

HCV-1   4591 ACG CCC GCC GAG ACT ACA GTT AGG CTA
             Thr Pro Ala Glu Thr Thr Val Arg Leu

HCV-1   4618 CGA GCG TAC ATG AAC ACC CCG GGG CTT
             Arg Ala Tyr Met Asn Thr Pro Gly Leu

HCV-1   4645 CCC GTG TGC CAG GAC CAT CTT GAA TTT
             Pro Val Cys Gln Asp His Leu Glu Phe

HCV-1   4672 TGG GAG GGC GTC TTT ACA GGC CTC ACT
             Trp Glu Gly Val Phe Thr Gly Leu Thr

HCV-1   4699 CAT ATA GAT GCC CAC TTT CTA TCC CAG
             His Ile Asp Ala His Phe Leu Ser Gln

HCV-1   4726 ACA AAG CAG AGT GGG GAG AAC CTT CCT
             Thr Lys Gln Ser Gly Glu Asn Leu Pro

HCV-1   4753 TAC CTG GTA GCG TAC CAA GCC ACC GTG
             Tyr Leu Val Ala Tyr Gln Ala Thr Val

HCV-1   4780 TGC GCT AGG GCT CAA GCC CCT CCC CCA
             Cys Ala Arg Ala Gln Ala Pro Pro Pro

HCV-1   4807 TCG TGG GAC CAG ATG TGG AAG TGT TTG
             Ser Trp Asp Gln Met Trp Lys Cys Leu

HCV-1   4834 ATT CGC CTC AAG CCC ACC CTC CAT GGG
             Ile Arg Leu Lys Pro Thr Leu His Gly

HCV-1   4861 CCA ACA CCC CTG CTA TAC AGA CTG GGC
             Pro Thr Pro Leu Leu Tyr Arg Leu Gly
```

FIG. 16-4

```
HCV-1   4888 GCT GTT CAG AAT GAA ATC ACC CTG ACG
             Ala Val Gln Asn Glu Ile Thr Leu Thr

HCV-1   4915 CAC CCA GTC ACC AAA TAC ATC ATG ACA
             His Pro Val Thr Lys Tyr Ile Met Thr

HCV-1   4942 TGC ATG TCG GCC GAC CTG GAG GTC GTC
             Cys Met Ser Ala Asp Leu Glu Val Val

HCV-1   4969 ACG AGC ACC TGG GTG CTC GTT GGC GGC
             Thr Ser Thr Trp Val Leu Val Gly Gly

HCV-1   4996 GTC CTG GCT GCT TTG GCC GCG TAT TGC
             Val Leu Ala Ala Leu Ala Ala Tyr Cys

HCV-1   5023 CTG TCA ACA GGC TGC GTG GTC ATA GTG
             Leu Ser Thr Gly Cys Val Val Ile Val

HCV-1   5050 GGC AGG GTC GTC TTG TCC GGG AAG CCG
             Gly Arg Val Val Leu Ser Gly Lys Pro

                                         Glu Val Leu
                                         GAA GTC CTC
C200
HCV-1   5077 GCA ATC ATA CCT GAC AGG --- --- ---
             Ala Ile Ile Pro Asp Arg

             Tyr Arg Glu Phe Asp Glu Met Glu Glu
C200    5104 TAC CGA GAG TTC GAT GAG ATG GAA GAG
HCV-1        --- --- --- --- --- --- --- --- ---

             Cys Ala Ser His Leu Pro Tyr Ile Glu
C200    5131 TGC GCC TCA CAC CTC CCC TAC ATC GAA
HCV-1        --- T-T CAG --- T-A --G --- --- --G
             Ser Gln
```

FIG. 16-5

```
                    Gln Gly Met Gln Leu Ala Glu Gln Phe
C200      5158      CAG GGA ATG CAG CTC GCC GAG CAA TTC
HCV-1               --A --G --- AT- --- --- --- --G ---
                              Met

                    Lys Gln Lys Ala Leu Gly Leu Leu Gln
C200      5185      AAG CAG AAG GCG CTC GGG TTG CTG CAA
HCV-1               --- --- --- --C --- --C C-C --- --G


                    Thr Ala Thr Lys Gln Ala Glu Ala Ala
C200      5212      ACA GCC ACC AAG CAA GCG GAG GCT GCT
HCV-1               --C --G T-- CGT --G --A --- -T- ATC
                              Ser Arg               Val Ile


                    Ala Pro Cys Glu Ser Met His Ala Ser
C200      5239      GCT CCG TGT GAG TCA ATG CAC GCC TCG
HCV-1               --C --T GC- -TC CAG -CC A-- TGG CAA
                              Ala Val Gln Thr Asn Trp Gln

C200      5266      A
HCV-1               -AA CTC GAG ACC TTC TGG GCG AAG CAT
                    Lys Leu Glu Thr Phe Trp Ala Lys His

HCV-1     5293      ATG TGG AAC TTC ATC AGT GGG ATA CAA TA
                    Met Trp Asn Phe Ile Ser Gly Ile Gln
```

FIG. 16-6

SUBSTITUTE SHEET

SUBSTITUTE SHEET

NS1 Sequence vs. HCV-1

```
                  Leu Gly Asn Trp Phe Gly Cys Thr Trp
J1          1     G TTG GGC AAT TGG TTC GGT TGC ACC TGG
HCV-1             - C-- --- --- --- --- --- --T --- ---

                  Met Asn Ser Ser Gly Phe Thr Lys Val
J1         29     ATG AAC TCA TCT GGA TTT ACC AAA GTG
HCV-1             --- --- --- A-- --- --C --- --- ---
                              Thr

                  Cys Gly Ala Pro Pro Cys Val Ile Gly
J1         56     TGC GGA GCG CCT CCT TGT GTC ATC GGA
HCV-1             --- --- --- --- --- --- --- --- ---
Ala

                  Gly Val Gly Asn Asn Thr Leu Gln Cys
J1         83     GGG GTG GGC AAC AAC ACC TTG CAA TGC
HCV-1             --- -C- --- --- --- --- C-- --C ---
                      Ala                         His

                  Pro Thr Asp Cys Phe Arg Lys His Pro
J1        110     CCC ACT GAC TGT TTC CGC AAG CAT CCG
HCV-1             --- --- --T --- --- --- --- --- ---

                  Asp Ala Thr Tyr Ser Arg Cys Gly Ser
J1        137     GAC GCC ACA TAC TCT CGG TGC GGT TCC
HCV-1             --- --- --- --- --- --- --- --C ---

                  Gly Pro Trp Ile Thr Pro Arg Cys Leu
J1        164     GGT CCC TGG ATT ACG CCC AGG TGC CTG
HCV-1             --- --- --- --C --A --- --- --- ---

                  Val His Tyr Pro Tyr Arg Leu Trp His
J1        191     GTC CAC TAC CCT TAT AGG CTT TGG CAT
HCV-1             --- G-- --- --G --- --- --- --- ---
                      Asp

                  Tyr Pro Cys Thr Val Asn Tyr Thr Leu
J1        218     TAT CCC TGT ACT GTC AAC TAC ACC TTG
HCV-1             --- --T --- --C A-- --- --- --- A-A
                                  Ile                 Ile
```

FIG. 17-1

		Phe	Lys	Val	Arg	Met	Tyr	Val	Gly	Gly
J1	245	TTC	AAA	GTC	AGG	ATG	TAC	GTG	GGA	GGG
HCV-1		--T	---	A--	---	---	---	---	---	---
				Ile						
		Val	Glu	His	Arg	Leu	Glu	Val	Ala	Cys
J1	272	GTC	GAG	CAC	AGG	CTG	GAA	GTT	GCT	TGC
HCV-1		---	--A	---	---	---	---	-C-	--C	---
								Ala		
		Asn	Trp	Thr	Arg	Gly	Glu	Arg	Cys	Asp
J1	299	AAC	TGG	ACG	CGG	GGC	GAG	CGT	TGT	GAT
HCV-1		---	---	---	---	---	--A	---	--C	---
		Leu	Asp	Asp	Arg	Asp				
J1	326	CTG	GAC	GAC	AGG	GAC	A			
HCV-1		---	--A	---	---	---	--			
			Glu							

FIG. 17-2

Core Sequence vs. HCV-1

```
J1          1    GCGTCTAGCCATGGCGTTAGTATGAGTGTC
HCV-1            ------------------------------

J1         31    GTGCAGCCTCCAGGACCCCCCCTCCCGGGAGAGCC
HCV-1            -----------------------------------

J1         66    ATAGTGGTCTGCGGAACCGGTGAGTACACCGGAAT
HCV-1            -----------------------------------

J1        101    TGCCAGGACGACCGGGTCCTTTCTTGGATCAACCC
HCV-1            -----------------------------------

J1        136    GCTCAATGCCTGGAGATTTGGGCGTGCCCCCGCGA
HCV-1            ----------------------------------A-

J1        171    GACTGCTAGCCGAGTAGTGTTGGGTCGCGAAAGGC
HCV-1            -----------------------------------

J1        206    CTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGC
HCV-1            -----------------------------------

                                              Met Ser
J1        241    CCCGGGAGGTCTCGTAGACCGTGCATCATG AGC
HCV-1            ------------------------C--------

                 Thr Asn Pro Lys Pro Gln Arg Lys Thr
J1        274    ACA AAT CCT AAA CCT CAA AGA AAA ACC
HCV-1            --G --- --- --- --- --- -A- --- -A-
                                         Lys     Asn

                 Lys Arg Asn Thr Asn Arg Arg Pro Gln
J1        301    AAA CGT AAC ACC AAC CGC CGC CCA CAG
HCV-1            --- --- --- --- --- --T --- --- ---

                 Asp Val Lys Phe Pro Gly Gly Gly Gln
J1        328    GAC GTC AAG TTC CCG GGC GGT GGT CAG
HCV-1            --- --- --- --- --- --T --C --- ---

                 Ile Val Gly Gly Val Tyr Leu Leu Pro
J1        355    ATC GTT GGT GGA GTT TAC CTG TTG CCG
HCV-1            --- --- --- --- --- --- T-- --- ---

                 Arg Arg Gly Pro Arg Leu Gly Val Arg
J1        382    CGC AGG GGC CCC AGG TTG GGT GTG CGC
HCV-1            --- --- --- --T --A --- --- --- ---
```

FIG. 18-1

```
                        Ala Thr Arg Lys Thr Ser Glu Arg Ser
J1          409         GCG ACT AGG AAG ACT TCC GAG CGG TCG
HCV-1                   --- --G --A --- --- --- --- --- ---

                        Gln Pro Arg Gly Arg Arg Gln Pro Ile
J1          436         CAA CCT CGT GGA AGG CGA CAA CCT ATC
HCV-1                   --- --- --A --T --A --T --G --- ---

                        Pro Lys Ala Arg Gln Pro Glu Gly Arg
J1          463         CCC AAG GCT CGC CAG CCC GAG GGC AGG
HCV-1                   --- --- --- --T -G- --- --- --- ---
                                        Arg

                        Ala Trp Ala Gln Pro Gly Tyr Pro Trp
J1          490         GCC TGG GCT CAG CCC GGG TAC CCT TGG
HCV-1                   A-- --- --- --- --- --- --- --- ---
                        Thr

                        Pro Leu Tyr Gly Asn Glu Gly Met Gly
J1          517         CCC CTC TAT GGC AAC GAG GGC ATG GGG
HCV-1                   --- --- --- --- --T --- --- TGC ---
                                                    Cys

                        Trp Ala Gly Trp Leu
J1          544         TGG GCA GGA TGG CTC CT
HCV-1                   --- --G --- --- --- --
```

FIG. 18-2

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05242

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C07H 21/04; C12Q 1/68
U.S. CL.: 536/27; 435/6

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	536/27; 435/6, 235.1; 424/89

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Databases: Dialog (Files: Medline, Biosis, Chem. Abstracts, World Patents index)
Automated patent Searching (1975-1991)

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	EP, A, 0,318,216 (Houghton et al) 31 May 1989, see the Abstract, pages 9 and claims 1-22.	1-8,28
Y	EP, A, 0,293,274 (Takahashi et al) 30 November 1988, see the Abstract.	1-8,28
Y	US, A, 4,428,941 (Galibert et al.) 31 January 1984, see column 4 lines 26-54.	1-8
Y,P	US, A, 4,870,026 (Wands et al.) 26 September 1989, see the Abstract.	1-8,28
A	US, A, 4,673,634 (Seto et al.) 16 June 1987.	1-8

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
28 January 1991	20 FEB 1991
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA?US	R. Keith Baker, Ph.D. ebw

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

See attachment.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1-8 and 28

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Attachment to PCT/ISA/210
VI. Observations Where Unity of Invention is Lacking

Group I, claims 1-8 and 28 are drawn to various DNA sequences for HCV isolates and a method of their use, classified in Classes 536 and 435, subclasses 27 and 6 respectively.

Group II, claims 9-16 are drawn to polypeptides derived from HCV;

Group III, claims 29-30 drawn to method of their expression;

Group IV, claims 17-20 drawn to method of their use in an Immunoassay.

Group V, claims 21-23 are drawn to against HCV isolates;

Group VI, claim 27 drawn to a method of making antibodies;

Group VII, claims 24-26 drawn to a method of using the antibodies.

DETAILED REASONS FOR HOLDING LACK OF UNITY OF INVENTION:

PCT Rule 13.2 permits claims to "a" (one) product and "a" (one) method of making and "a" (one method of using said product. The invention as set forth in Group I constitutes a combination of a product and a method of its use. The inventions as defined by Groups II and V are drawn to two additional products, Groups III and VI are drawn to additional methods of their preparation and Groups IV and VII are drawn to other methods of their use. The claimed DNA sequences, polypeptides and antibodies are each structurally and functionally distinct from each other and each group requires a different field of search.